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POLYPHENOLIC PROFILE AND ANTIMICROBIAL POTENTIAL OF PEEL EXTRACTS OBTAINED FROM ORGANIC POMEGRANATE (*PUNICA GRANATUM* L.) VARIETY "MOLLAR DE ELCHE"

Marina CANO-LAMADRID*, Manuel VIUDA-MARTOS, José Miguel GARCÍA-GARVÍ, Jesús CLEMENTE-VILLALBA, Ángel A. CARBONELL-BARRACHINA, Esther SENDRA

Universidad Miguel Hernández, Orihuela, Spain

The aim of this work was to determine the polyphenolic profile and the antibacterial properties of extracts from organic pomegranate peel, to evaluate if it could be used as a potential antimicrobial ingredient to elaborated organic food. The antibacterial properties of different organic pomegranate peel extracts (ethyl acetate, acetone and methanol, with an increasing polarity) were tested against: *Listeria innocua*, *Achromobacter denitrificans* and *Algaligenes faecalis*. All extracts showed antimicrobial activity against all bacteria tested except ethyl acetate extract against *A. denitrificans*. The polyphenolic profile was determined by High Performance Liquid Chromatography (HPLC). Five compounds were identified. Punicagalin was the main component found in acetone and methanol extracts (7,939 $\mu g.g^{-1}$ and 5,178 $\mu g.g^{-1}$ of lyophilized sample, respectively). Ellagic acid was the main component found in ethyl acetate extract (171 $\mu g.g^{-1}$ of lyophilized sample).

Keywords: antibacterial activity, ellagic acid, punicalagin, polyphenolic compounds

Consumers have been questioning the safety of synthetic preservatives of food (Al-Zoreky, 2009). As a result, there is an increasing demand for natural products which could serve as alternative food preservatives (Tajkarimi, Ibrahim, and Cliver, 2010; Tehranifar et al., 2011). One of them could be the co-products generated in the industrial transformation of vegetables and fruits, insomuch as these are potentially good sources of bioactive compounds (Ayala-Zavala et al., 2011). In this way, during the industrial processing of pomegranate juice, the wastes (mainly peel and internal membranes) on average account for 50% of the fresh weight. In the last recent years, little attention has been paid to pomegranate non-edible parts (Hasnaoui, Wathelet, and Jiménez-Araujo, 2014). Pomegranate peel is a rich source of polyphenolic compounds, which possess antibacterial properties (Al-Zoreky, 2009; Agourram et al., 2013). A linear relationship between total phenolic content and antibacterial activity against several microorganisms has been previously reported (Shan et al., 2007).

In our knowledge, this is the first study where the polyphenolic profile of organic certified pomegranate peel extracts and their antimicrobial properties are evaluated against listeria and other bacteria from refrigerated foods. Thus, the aim of this work was to determine the polyphenolic profile and the antibacterial properties of different organic pomegranate peel extracts (acetone, methanol and ethyl acetate based extracts), to know if they could be used as potential antimicrobial ingredients to be included in organic foods.

Material and method

Plant material

Ripe pomegranate fruits, variety "Mollar de Elche", were obtained from an organic field certified by CAERM (Council Agriculture the Ecological Region of Murcia) in Alquerias (Murcia). Pomegranate peel (PoP) was obtained following the procedure described by Gullón et al. (2016). After that, the sample was lyophilized and stored under vacuum until analyzed.

Determination of polyphenolic compounds

Extraction of polyphenolic compounds

The method run was described by Gullón et al. (2016). The solvents used were: ethyl acetate (ea), 70% acetone in water (ac) and 80% methanol in water (m) with an increasing polarity. Three replicates were performed for each extraction in order to calculate extraction yield, which was calculated after removing the extraction solvents. Extracts were named: PoP methanol (PoPm), PoP acetone (PoPac) and PoP ethyl acetate (PoPea).

HPLC analysis

The determination of the phenolic compounds using High Performance Liquid Chromatography (HPLC) was performed on a Hewlett-Packard HPLC series 1,200 instrument (Woldbronn, Germany) equipped with UV-Vis Diode Array

Contact address: Marina Cano-Lamadrid, Research Personel, Universidad Miguel Hernández de Elche, Departamento Tecnología Agroalimentaria, Grupo Calidad y Seguridad Alimentaria, Carretera de Beniel, km 3.2, Orihuela 03312, Spain, ☎ 966749735, e-mail: marina.cano.umh@gmail.com

Detector following Gullón et al. (2016). The compounds were quantified through calibration curves of standard compounds as mean of three replicates.

Antimicrobial activity

Microorganisms and growth conditions

Each pomegranate peel extract (PoPx) were was individually tested against bacterial strains which are common spoilage agents or indicators of the presence of pathogenic bacteria in refrigerated foods: *Listeria innocua* CECT 910, *Achromobacter denitrificans* CECT 449 and *Alcaligenes faecalis* CECT 145. These species were supplied by the Spanish Type Culture Collection of the University of Valencia (CECT).

Minimum inhibitory concentration (MIC)

The antimicrobial activity against bacteria species was determined based on a method proposed by Abate, Mshana, and Miorner, (1998), with some modifications. Bacteria were cultured in Mueller Hinton (MH) broth for 24 h at 37 ºC (*L. innocua*, *A. denitrificans* and *A. faecalis*) and diluted in sterile MH broth to a final level of 10^6 colony forming units (CFU) mL^{-1}. Dried organic PoPx obtained with ethyl acetate, acetone 70% and methanol 80% were suspended to concentration ranges from 50 to 100 $mg.mL^{-1}$ in MH and sterilized by filtration though a 0.22 μm nylon filter. The 96-well microplates (Iwaki, Japan) were prepared by dispensing the extracts (the concentration of the extracts on the media tested on the microplates ranged from 20 microliters/mL to 0.009 $microliters.mL^{-1}$), fresh media and 20 μL of microbial suspension (containing about 10^6 $CFU{\cdot}mL^{-1}$) to a total volume of 300 μL. Contents of each well were mixed on a plate shaker at 150 rpm for 2 min prior to incubation for 24 h at 37 ºC. After incubation, 25 μL of 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide (MTT) (Sigma Life Science), dissolved in dimethyl sulfoxide (DMSO) was added to each of the wells and incubated for 1 h in order to allow the viable microorganisms to metabolize the yellow MTT dye into formazan (purple crystals). The MIC was the concentration of the first well that did not undergo colour change (from yellow to purple). Results were confirmed by plating 10 mL samples from clear wells onto MH agar medium. The procedure was repeated three times for each microorganism.

Well diffusion method

The antibacterial action of the PoPx (100 $mg.mL^{-1}$) was tested on bacteria described using the well diffusion method (Smith-Palmer, Stewart, and Fyfe, 1998). Wells were aseptically made in the agar at the center of the plate, in order to add 100 μL of the extract. The inhibition zones around the wells were measured using a transparent millimeter ruler. Two zones were identified as bactericidal and bacteriostatic (Smith-Palmer, Stewart, and Fyfe, 1998). For each extract and microorganism tested, three replicates were made.

Statistical assay

Results are provided as the mean ± standard error. First, data was subjected to one-way (factor = extract) analysis of variance (ANOVA) and later, data was also subjected to the Tukey's multiple-range test to compare the means. Differences were considered statistically significant at p <0.05. All statistical analyses were performed using StatGraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD).

Results and discussion

Polyphenolic profile

The extraction yield of the extracts was in a decreasing order: Acetone > Methanol > Ethyl acetate (86.5, 67.5 and 3.8%, respectively). The effect of the solvent used for extraction showed significant differences (p <0.05) on the extraction yield. In most of published researches on phenolic extraction (Al-Zoreky, 2009; Akhtara et al., 2015; Nuncio-Jáuregui et al., 2015), the solvent used was methanol or methanol-water given their high extraction yield, however, in this work the most effective solvent was acetone-water, although methanol extraction yield was also high. Table 1 shows the HPLC profile of the three PoPx. A total of five phenolic compounds were identified as punicalagins (PC), ellagic acid (EA) and ellagic acid derivatives (EAd_1, EAd_2 and EAd_3) in all extracts except EAd_1 which was not detected in PoPea. In this work, PC is the sum of both isomers (α-punicalagin and β-punicalagin) as usually reported by other authors (Calín-Sánchez et al., 2013; Nuncio-Jáuregui et al., 2015).

Table 1 Polyphenolic profile of organic pomegranate peel extracts (PoPx)

Compound	Lyophilized Organic PoPx (μg.g^{-1})		
	PoPm	PoPac	PoPea
Punicalagin	5,177 ±249^{b}*	7,939 ±103^{a}	14 ±5^{c}
Ellagic acid	603 ±12^{b}	1,026 ±17^{a}	171 ±3^{c}
Ellagic acid derivate 1	823 ±80^{b}	1,026 ±12^{a}	nd**
Ellagic acid derivate 2	293 ±8^{b}	494 ±4^{a}	20 ±2^{c}9
Ellagic acid derivate 3	283 ±10^{b}	528 ±6^{a}	22 ±8^{c}

PoPm – 80% methanol extract; PoPac – 70% acetone extract; PoPea – ethyl acetate extract; * – values followed by different letter within the same line were statistically different according to the Tukey's multiple range test, (p <0.05); nd** – below LOQ [limit of quantification determined as three times the standard deviation of the blanks, limit of detection (LOD), multiplied by the proper dilution factor]

Table 2 Minimum inhibitory concentration (MIC) of organic pomegranate peel extracts (PoPx) against *L. innocua*, *A. faecalis* and *A. denitrificans*

Microorganism	MIC (mg.mL^{-1})		
	PoPm	PoPac	PoPea
***Listeria innocua* CECT 910**	50*	50	50
***Alcaligenes faecalis* CECT 145**	70^{b}	70^{b}	100^{a}
***Achromobacter denitrificans* CECT 449**	100^{a}	80^{b}	NA**

PoPm – 80% methanol extract; PoPac – 70% acetone extract; PoPea – ethyl acetate extract; * – values followed by different letter within the same line were statistically different according to the Tukey's multiple range test (p <0.05); NA** – not active

Table 3 Antibacterial effects of PoPx (100 mg.mL^{-1}) on each bacteria studied, applying the diffusion method

Microorganism	Diameter of inhibition zone (mm) *		
	PoPm	PoPac	PoPea
***Listeria innocua* CECT 910**	2.4±0.2 (1.2±0.1)	1.9±0.3 (1.4 ±0.1)	1.4±0.1 (0.9±0.1)
***Alcaligenes faecalis* CECT 145**	nd**	nd	nd
***Achromobacter denitrificans* CECT 449**	nd	nd	nd

* – the values shown are the mean averages for three replications; ** – no inhibition detected; Values within brackets indicate bactericidal effect (no growth), whereas plain text valuers indicate bacteriostatic effect (slight growth detected)

The results showed that PoPac had the highest (p <0.05) contents of all phenolic compounds identified followed by PoPm. The major compound found in PoPm and PoPac was PC (p <0.05) while in PoPea it was EA. The results obtained were similar that to those reported by other authors (Sarkhosh et al., 2007; Lu, Ding, and Yuan, 2008; Gullón et al., 2016) who mentioned that the most abundant polyphenolic compounds in pomegranate peel were PC followed by EA.

However, the use of organic solvents in the manufacturing process of food ingredients, in general and in organic food in particular, is regulated and environmental aspects should be taken into account related of the use of them as the solvents used for extraction (Tabaraki, Heidarizadi and Benvidi, 2012), in this case acetone and methanol are both biodegradable.

MIC determination

The MICs, expressed in mg.mL^{-1}, of the extracts are presented in Table 2. All extracts showed antimicrobial activity against all bacteria tested except PoPea against *A. denitrificans*. *L. innocua* was inhibited in the presence of 50 mg.mL^{-1} of all PoPx. No statistical differences were found (p >0.05) in MICs values of PoPac and PoPm against *A. faecalis*, whilst MIC of PoPea was 100 mg.mL^{-1} (p <0.05). As regards, *A. denitrificans*, PoPea was not effective. In general, the most active extract was PoPac (p <0.05) followed by PoPm.

Inhibitory activity of PoPx against the microorganisms may due to polyphenolic compounds present in the extracts, mainly punicalagins and ellagic acid or their derivatives. High-molecular weight proteins may react with polyphenolic compounds and constitute complex molecules, which can react with oxyreductase (cellular enzymes) that exists in cell walls of bacteria (Tehranifar et al., 2011), impairing the cell membrane structure, leading to a loss of cell homeostasis (Li et al., 2014). There are several works which inform that these compounds have been recorded as having anti-microbial activity (Reddy et al., 2007; Shan et al., 2007; Abuelsaad et al., 2013; Agourram et al., 2013): EA has been reported to have antibacterial activity against both Gram-positive and Gram-negative pathogens (Miguel, Neves, and Antunes, 2010).

Diffusion method

The antimicrobial efficacy of the PoPx against bacteria was evaluated using a diffusion method to measure the surrounding inhibition zones. Table 3 shows the antibacterial activity of the PoPx (100 µL concentration 100 mg.mL^{-1}) as determined by applying the diffusion method. All extracts were effective against *L. innocua* being the maximum value for bacteriostatic effect 2.4 mm which corresponded to PoPm and really scarce for bactericidal effect. As to the other bacteria, both Gram negative, no inhibitory effect was observed using the studied extractsstudied. Given that the inhibitory effect was only detected against the only Gram positive bacteria tested, it may be possible that the nature of the cell wall would be related to the mechanism of action of the extracts. These results showed as well, that compounds in the extracts have little diffusivity to the surrounding environment and that their mechanisms of inhibitory action require the direct contact with the target bacteria. So they would be effective when included in food formulation as well as in antimicrobial coatings or films.

Conclusions

From the present study, it could be concluded that organic pomegranate peel has antimicrobial activity due to the content of polyphenolic compounds. The polar solvent acetone : water (70 : 30) gets the highest extraction yield as well as antimicrobial activity. Pomegranate peel extracts grown by organic agricultural practices could be considered as a possible functional source of additives exerting antibacterial effect against listeria and other psicrophilic psychrophilic bacteria to elaborate organic foods or to be included in films or coatings.

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References

ABATE, G. – MSHANA, R. N. – MIORNER, H. 1998. Evaluation of a colorimetric assay based on 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide (MTT) for rapid detection of rifampicin resistance in Mycobacterium tuberculosis. In International Journal of Tuberculosis and Lung Disease, vol. 2, 1998, pp. 1011–1016.

ABUELSAAD, A. S. A. – MOHAMED, I. – ALLAM, G. – AL-SOLUMANI, A. A. 2013. Antimicrobial and inmunomodulating activities of hesperidin and ellagic acid against *Aeromonas hydrophila* in a murine model. In Life Sciences, vol. 93, 2013, pp. 714–722.

AGOURRAM, A. – GHIRARDELLO, D. – RANTSIOU, K. – ZEPPA, G. – BELVISO, S. – ROMANE, A. – OUFDOU, K. – GIORDANO, M. 2013. Phenolic content, antioxidant potential and antimicrobial activities of fruit and vegetables by-products extracts. In International Journal of Food Properties, vol. 16, 2013, no. 5, pp. 1092–1104.

AKHTARA, S. – ISMAIL, T. – FRATERNALE, D. – SESTILI, P. 2015. Pomegranate peel and peel extracts: Chemistry and food features. In Food Chemistry, vol. 174, 2015, pp. 417–425.

AL-ZOREKY, N.S. 2009. Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. In International Journal of Food Microbiology, vol. 134, 2009, pp. 244–248.

AYALA-ZAVALA, J. – VEGA-VEGA, V. – ROSAS-DOMÍNGUEZ, C. – PALAFOX-CARLOS, H. – VILLA-RODRÍGUEZ, J. – SIDDIQUI, M. W. – DÁVILA-AVILA, J.E. – GONZÁLEZ-AGUILAR, G.A. 2011. Agro-industrial potential of exotic fruit byproducts as a source of food additives. In Food Research International, vol. 44, 2011, no. 7, pp. 1866–1874.

CALÍN-SÁNCHEZ, A. – FIGIEL, A. – HERNÁNDEZ, F. – MELGAREJO, P. – LECH, K. – CARBONELL-BARRACHINA, A. A. 2013. Chemical composition, antioxidant activity and sensory quality of pomegranate (*Punica granatum* L.) arils and rind as affected by drying method. In Food and Bioprocess Technology, vol. 6, 2013, pp. 1644–1654.

GULLÓN, B. – PINTADO, M. – PEREZ-ALVAREZ, J. A. – VIUDA-MARTOS, M. 2016. Assessment of polyphenolic profile and antibacterial activity of pomegranate peel (*Punica granatum*) flour obtained from co-product of juice extraction. In Food Control, vol. 59, 2016, pp. 94–98.

HASNAOUI, N. – WATHELET, B. – JIMÉNEZ-ARAUJO, A. 2014. Valorization of pomegranate peel from 12 cultivars: Dietary fiber composition, antioxidant capacity and functional properties. In Food Chemistry, vol. 160, 2014, pp. 196–203.

LI, G. – XU, Y. – WANG, X. – ZHANG, B. – SHI, C. – ZHANG, W. – XIA, X. 2014. Tannin-rich fraction from pomegranate rind damages membrane of *Listeria monocytogenes*. Foodborne. In Pathogens and Disease, vol. 11, 2014, no. 4, pp. 1–7.

LU, J. – DING, K. – YUAN, Q. 2008. Determination of punicalagin isomers in pomegranate husk. In Chromatographia, vol. 68, 2008, pp. 303–306.

MIGUEL, M. – NEVES, M. – ANTUNES, M. 2010. Pomegranate (*Punica granatum* L.): a medicinal plant with myriad biological properties-a short review. In Journal of Medicinal Plants Research, vol. 4, 2010, pp. 2836–2847.

NUNCIO-JÁUREGUI, N. – MUNERA-PICAZO, S. – CALÍN-SÁNCHEZ, A. – WOJDYLO, A. – HERNÁNDEZ F. – CARBONELL-BARRACHINA, A. A. 2015. Bioactive compound composition of pomegranate fruits removed during thining. In Journal of Food Composition and Analysis, vol. 37, 2015, pp. 1–19.

REDDY, M.K. – GUPTA, S.K. – JACOB, M.R. – KHAN, S.L. – FERRIRA, D. 2007. Antioxidant, antimalarial ad antimicrobial activities of tannin-rich fractions, elagitannins and phenolic acid from *Punica granatum* L. In Planta Medica, vol. 73, 2007, no. 5, pp. 461–467.

SARKHOSH, A. – AMANI, Z. – FATAHI, R. – GHORBANI, H. – HADIAN, J. 2007. A review on medicinal characteristic of pomegranate (*Punica granatum* L.). In Journal of Medicinal Plants Research, vol. 6, 2007, no. 22, pp. 13–24.

SHAN, B. – CAI, Yi-Z. – BROOKS, J.D. – CORKE, H. 2007. The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts. In International Journal of Food Microbiology, vol. 117, 2007, no. 1, pp. 112–119.

SMITH-PALMER, A. – STEWART, J. – FYFE, L. 1998. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. In Letters in Food Microbiology, vol. 26, 1998, pp. 118 – 122.

TABARAKI, R. – HEIDARIZADI, E. – BENVIDI, A. 2012. Optimization of ultrasonic-assisted extraction of pomegranate (*Punica granatum* L.) peel antioxidants by response surface methodology. In Separation and Purification Technology, vol. 98, 2012, pp. 16–23.

TAJKARIMI, M. – IBRAHIM, S. – CLIVER, D. 2010. Antimicrobial herb and spice compounds in food. In Food Control, vol. 21, 2010, no. 9, pp. 1199–1218.

TEHRANIFAR, A. – SELAHVARZI, Y. – JHARRAZI, M. – BAJHSG, V.J. 2011. High potential of agro-industrial by-products of pomegranate (*Punica granatum* L.) as the powerful antifungal and antioxidant substances. In Industrial Crops and Products, vol. 34, 2011, pp. 1523–1527.

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SWEET CHERRY FRUIT CHARACTERISTIC IN COVERED ORCHARDS

Radek VÁVRA

Research and Breeding Institute of Pomology Holovousy Ltd., Czech Republic

The aim of evaluations performed in experimental plantings of the Research and Breeding Institute of Pomology Holovousy Ltd. was to verify the effect of covering systems on fruit characteristics – size, weight, firmness and soluble solids content (SSC). The research was focused on evaluation of fruit characteristics in 2017 and 2018 on fourteen cultivars: ´Amid´, ´Cristiana´, ´Early Korvik´, ´Elza´, ´Fabiola´, ´Felicita´, Horka´, ´Justyna´, ´Kasandra´, ´Kordia´, ´Korvik´, ´Tamara´, ´Těchlovan´ and ´Vanda´. The tested cultivars were grown in an IPM irrigated covered orchard. The largest fruit size was recorded for the cultivar ´Tamara' with 29.5 mm followed by the cultivar ´Horka' with 29 mm and ´Felicita´ with 28.6 mm. The greatest weight was recorded for the cultivar ´Tamara' with 12.4 g followed by ´Felicita´ with 12.3 g. The greatest firmness was recorded in fruits of the cultivar ´Amid´ with index 77.5 and ´Tamara´ with index 73.2. High correlation between fruit characteristics was found only between weight and size of fruits (correlation coefficient 0.88). High differences were recorded between years. In 2017, fruits characteristics were higher (size 27.5 mm, weight 10.9 g, firmness 65.3, SSC 19.3 °Brix) while in 2018, fruit characteristics were lower (size 26.5 mm, weight 9.2 g, firmness 59.2, SSC 16.5 °Brix).

Keywords: *Prunus avium* L.; fruit quality; fruit weight; fruit size; fruit firmness; soluble solid content

Modern sweet cherry orchards are equipped with covering systems against rain which affect the quality of the fruit in particular by reducing the rain-induced fruit cracking. The positive effect of covered plantings is also higher fruit size and weight which increases the price of cherries and marketability of production. Nowadays, retail chains and consumers demand high-sized cherries over 30 mm in the width of the fruits (Measham et al., 2014, Meland et al., 2014). This size can be achieved by using covering systems against rain. Information related to the specific conditions of growing cherries in covered plantings, supplemented with knowledge about technologies of protection against major diseases and pests is essential for cherry growers. Higher quality and better marketability production from covered plantings is proved by ever-increasing areas of these systems in Europe and in all other continents. The most commonly used systems in practice include Haygrove tunnels (www.haygrove.com), and single-row systems e.g. VOEN system (www.voen.de). Other systems, such as retractable roof technology (www.cravo.com), are also used in North and South America. Regardless of the type of covering systems, the main objective is to achieve the harvest of high market quality fruits. The influence of the VOEN system on the quality of cherries was evaluated at the location Holovousy in the Research and Breeding Institute of Pomology, Czech Republic. The results are presented in this paper.

Material and method

The research was focused on covered experimental cherry plantings at the location Holovousy. Climatic conditions of Holovousy are characterized by average annual temperature of 8.1°C and average annual rainfall of 655 mm. The soil was medium loam sandy with rather deep cultivated layer on gravel substrate. The orchard was located at the altitude of 300–370 masl. Experimental trees were trained as spindles using strong wooden stakes as supports. Clean strips were kept under the trees by contact herbicides whereas frequently cut sod was kept in alleys between the tree rows. Fertilizers were applied according to soil analyses. Spraying treatment against pests and diseases was conducted based on recommendations used for commercial orchards. The tested cultivars were grown in an IPM covered orchard. Irrigation was applied in the covered orchard. The experiments were focused on sweet cherry fruit characteristics.

The aim of the experiments performed in 2017–2018 was to verify the effect of covering systems (company VOEN, Germany) on cultivar differences in fruit weight, fruit size, fruit sweetness (soluble solids content; SSC) and fruit firmness. In this evaluation there were involved cultivars ´Amid', ´Early Korvik', ´Elza', ´Fabiola', ´Felicita', ´Horka', ´Christiana', ´Justyna', ´Kasandra', ´Kordia', ´Korvik', ´Tamara', ´Těchlovan' and ´Vanda'.

The fruits were harvested at the ripening time according to the individual cultivars. The samples were transferred to the laboratory and evaluated immediately. In each sample, 25 fruits were evaluated for each genotype in two replicates. Fruit weight in grams, fruit size (width in diameter) in millimetres, fruit soluble solids content (SSC) in Brix and fruit firmness were recorded. SSC was measured using a digital refractometer HI 96801 (HANNA Instruments, USA), fruit firmness by a Durofel instrument (Copa-Technology, France)

Contact address: Radek Vávra, Research and Breeding Institute of Pomology Holovousy Ltd., Holovousy 129, 508 01 Hořice, Czech Republic; e-mail: radek.vavra@vsuo.cz

by index of 1–100 (with 100 being the maximum firmness), weight on a digital scale Kern 440-49N (KERN & SOHN GmbH, Germany), and fruit width by digital callipers. All data were statistically processed by an analysis of variance by STATISTICA software (version 12, Stat Soft). Significant differences between the means for each sweet cherry cultivars were determined by the Tukey's test at p <0.05. The mutual dependence of two measured characteristics of individual fruits in all evaluated cultivars is expressed by correlation coefficients.

Table1 **Evaluation** of sweet cherry cultivars in 2017 and 2018

Cultivar	Weight (g)	Size (mm)	SSC (°Brix)	Firmness (index 1–100)
Amid	8.0 g	24.8 hi	19.1 ab	77.5 a
Cristiana	9.7 def	27.7 bcd	20.2 a	61.7 bcd
Early Korvik	10.0 def	27.0 de	17.4 bcd	58.7 cd
Elza	10.3 cde	27.3 cd	18.7 abc	66.3 b
Fabiola	10.5 cde	26.9 de	17.4 bcd	57.2 d
Felicita	12.3 ab	28.6 ab	15.1 e	64.6 bc
Horka	10.0 ab	29.0 ab	18.0 abcd	63.6 bc
Justyna	9.9 def	26.8 def	18.4 abc	59.0 cd
Kasandra	7.8 g	24.4 i	16.3 de	42.7 e
Kordia	10.6 cd	26.4 defg	19.0 ab	57.5 a
Korvik	9.5 ef	26.0 efg	17.1 cde	62.2 bcd
Tamara	12.4 a	29.5 a	18.3 abc	73.2 a
Těchlovan	11.1 bc	28.2 bc	17.2 cde	64.5 b
Vanda	9.1 f	25.8 gh	19.7 a	63.1 bc

Table 2 Correlation coefficients of fruit characteristics

Fruit characteristic	Weight	Size	SSC	Firmness
Weight	x	0.88	0.53	0.35
Size	0.88	x	0.33	0.38
SSC	0.53	0.33	x	0.33
Firmness	0.35	0.38	0.33	x

Table 3 Differences between years

Year	Weight (g)	Size (mm)	SSC (°Brix)	Firmness (index 1–100)
2017	10.9 a	27.5 a	19.3 a	65.3 a
2018	9.2 b	26.5 b	16.5 b	59.2 b

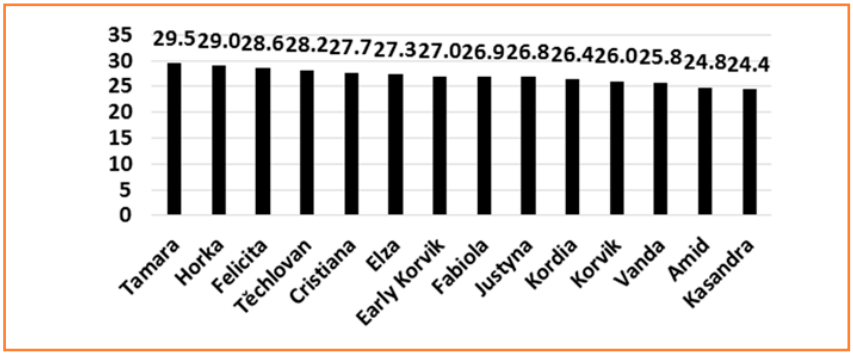

Figure 1 Evaluation of fruit size (mm)

Results and discussion

The results of the evaluation are shown in tables 1–3 and Figs 1–4.

Fruit size

Fruit size was higher in 2017 with the mean value of 27.5 mm than in 2018 with the mean value of 26.6 mm. The biggest fruits were recorded for the cultivar ´Tamara' (29.7 mm) followed by the cultivars ´Horka´ and ´Felicita´ with 29.0 mm and 28.6 mm, respectively.

Fruit weight

Fruit weight was higher in 2017 (10.9 g) than in 2018 (9.2 g). Year on year differences in the weight of the fruit were observed mainly in the cultivars ´Amid', ´Korvik' and ´Vanda'. The weight of the cultivar ´Amid' was by 25.1% lower in 2018 (6.8 g) than in 2017 (9.1 g). The same phenomenon was observed for ´Korvik' with the weight by 24.8% lower in 2018 (8.2 g) than in 2017 (10.9 g). The weight of the cultivar ´Vanda' was also lower by 25.2% in 2018 (7.8 g) than in 2017 (10.4 g). The greatest weight was recorded for the cultivar ´Horka' in 2017 (12.9 g). In both years, the cultivar ´Tamara' had the biggest fruit weight (12.4 g). In 2017, the smallest fruit weight was recorded by the cultivar ´Kasandra' (7.8 g).

Fruit firmness

Fruits were less firm (59.2) in 2018 than in 2017 (65.3). The greatest firmness was recorded for ´Tamara' in 2017 (82.7). In 2018, the highest fruit firmness was found for ´Amid' (74.5). On the contrary, the lowest firmness of 41.2 was recorded for the cultivar ´Kasandra' in 2018 and 44.2 in 2017.

SSC

Higher fruit sweetness was recorded in 2017 (19.3 °Brix) than in 2018 (16.5 Brix). This observation can be explained by hot weather during ripening in 2018. Fruits ripened very quickly and it had the impact on lower fruit sweetness and also fruit size (both weight and width).

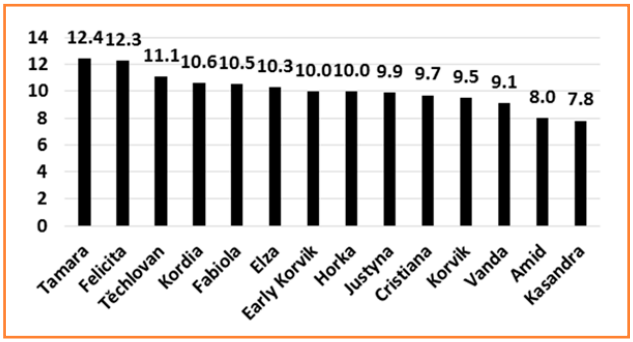

Figure 2 Evaluation of fruit weight (g)

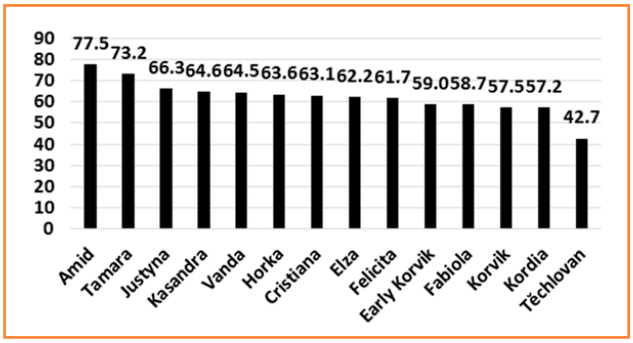

Figure 3 Evaluation of fruit firmness (index 1–100)

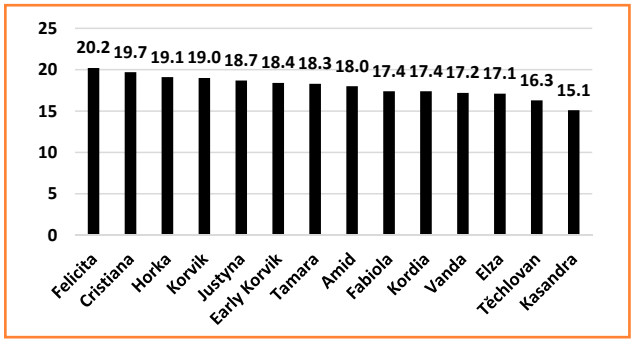

Figure 4 Evaluation of SSC (°Brix)

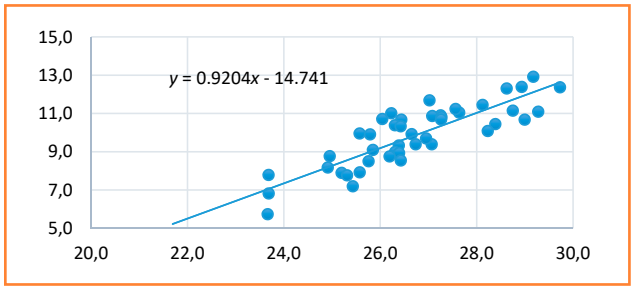

Figure 5 Dependence of fruit size and fruit weight

Correlation between fruit characteristics

High correlation between fruit characteristics (table 2) was found only between weight and width of fruits (correlation coefficient 0.88). This dependence can be described using the linear regression function $y = 0.9204x - 14.741$ (Figure 5). Medium dependence was found between the fruit weight and the fruit SSC (correlation coefficient 0.53). Low dependence was recorded in the relation fruit weight – fruit firmness (correlation coefficient 0.35), size – SSC (correlation coefficient 0.33) and size – firmness (correlation coefficient 0.38).

Conclusion

The results point to large year on year differences in fruit weight, size, SSC and firmness and also differences among the tested cultivars. In 2017, the fruits of all cultivars reached higher weight and fruit size than in 2018. The cause of the small yields in 2017 is frost damage during the tree flowering period; the fruit set was reduced. As a result of frost damage, the fruit set was lower in 2017 which was reflected in their higher weight and size. The lower weight and size of fruits in 2018 can be explained by very warm and dry weather, when the fruits of all cultivars ripened very quickly and the period was not long enough to grow to the size and weight as in 2017.

The records showed differences in fruit firmness between years and among the tested cultivars. Differences in fruit firmness among the cultivars indicate the differences in their cell structures, skin characteristics, compositions and/or respiration rates (Karacali, 2012). The highest sizes of fruits (29 mm and more) were achieved by the cultivars ´Tamara', ´Horka', and the newly registered cultivar ´Felicita'. The weight of fruits over 12 g was recorded in the cultivars ´Tamara', ´Horka' and ´Felicita', which confirms the high quality of the breeding programme of sweet cherries at Holovousy that is focused on selection and creation of high fruit size cherry cultivars. Fruit firmness of ´Amid´ and 'Tamara´ was higher than that of the other cultivars.

The differences in fruit characteristics among the cultivars observed in this evaluation of tested cultivars are in accordance with the observation of Sen et al., 2014 which includes differences in fruit quality during storage and transportation to consumers. Breeding programme and selection of new cultivars should be also focused on high ability of cherry fruits to long term storage and long shelf life.

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References

KARACALI, I. 2012. Storage and marketing of horticultural products. Izmir, Turkey : Ege University Agricultural Faculty Publication, 2012, no. 494.

MEASHAM, P.F. – GRACIE, A.J. – WILSON, S.J. – BOUND, S.A. 2014. An alternative view of rain/inducted cracking of sweet cherries (*Prunus avium* L.). In Acta Horticulturae, 2014, no. 1020, pp. 217–222.

MELAND, M. – KAISER, C. – CHRISTENSEN, J.M.2014. Physical and chemical methods to avoid fruit cracking in cherry. In AgroLife Scientifi c Journal, vol. 3, 2014, no. 1, pp. 177–183.

FATIH, S. – RUSTU, O. – GOLKARIAN, M et al. 2014. Quality Changes of Different Sweet Cherry Cultivars at Various Stages of the Supply Chain. In Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 42. 10.15835/nbha.42.2.959

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THE SAFFRON (*CROCUS SATIVUS* L.) CULTIVATION INTRODUCTION IN MOUNTAINOUS OASES OF ALGERIA

Khalila BENGOUGA*[1, 2], Selwa LAHMADI[1, 2], Rouguia ZEGUERROU[2], Moufida MAAOUI[2], Youcef HALIS[2]

[1]Mohamed Khider University, Biskra, Algeria
[2]Center of Scientific and Technical Research on Arid Regions (CRSTRA), Biskra, Algeria

An FNR project entitled "Promotion of Saffron (*Crocus sativus* L.) cultivation for the profit of arid and semi-arid regions' women" is led by the CRSTRA. The investigations made during 2013 and 2014 in different arid and semi-arid regions of Algeria have permitted to install Saffron (*Crocus sativus* L.) cultivation in five principle sites: Ain Zaatout, Beni Souik, Branis, Djemorah and Maafa (Algeria). These mountainous oases are characterised especially by lack of water. After analysing the investigation results, saffron corms were distributed to women to be cultivated. The sowing was realised on different dates. The results obtained in the first year of saffron cultivation in mountainous oases of Algeria are promising. The flowering took place in the same year in four sites; it is the case of Ain Zaatout, Beni Souik, Djemorah and Maafa. However, the flowering took place in the second year in the site of Branis. The difference in saffron flowering occurrence can be attributed to the difference in altitude, the pedo-climatic difference as well as the difference in the date of sowing.

Keywords: Saffron, mountainous oases, flowering, Algeria

Saffron has been a savoured flavour since antiquity, a well-known and highly priced dried spice which is obtained from the stigmas and the styles' tops of *Crocus sativus* L. The area of its repartition spreads from the Mediterranean Sea over Persia to India (Ait-Oubahou and El-Otmani, 1999; Schmidt and Betti, 2006). Thus, saffron growing can prosper without doubt in all North Africa; Algeria, Tunisia, and Morocco (Chevalier, 1926). Increasing world interest in the use of fragrance, colour, flavour and medicinal sources with plant origin, has broadened horizon for production and consumption of saffron (Behdani, 2011). It is a traditional production, practiced in Morocco for several centuries (Garcin and Carral, 2007) and it has been operated in Morocco for more than 15 years especially during 2004 (Lage, Faiz and Cantrell, 2006). Nowadays, a pioneering project has been monitored by the CRSTRA during 2009–2012 in Algeria (Lahamadi et al., 2013); the encouraging results of the first project led to the proposition of a second project which was conducted during 2013–2016, focusing on saffron introduction for the profit of rural women. Thus to improve rural population income, and to create and to valorise rural women's jobs; the multi-annual cultivation of saffron not very exigent in surface, water and mechanisation has been introduced in 5 mountainous oases of Algeria.

Material and method

After a prospection period during 2013–2014 in different arid and semi-arid regions of Biskra and Batna districts (Algeria: Fig. 1), an enquiry was conducted in five oases about the knowledge and the acceptance of the cultivation of saffron by rural women; samples consisted of 10% of the families owing land except for the oases of Djemorah where only two samples were inquired due to technical constraints. A woman per household was inquired whether she knew saffron (*Crocus sativus* L.) and if she accepted to cultivate the corms and keep up the growing until flowering.

Based on the results of the enquiry, 5 sites in the piedmonts of Aurès in North East of Biskra (Fig. 1) were chosen based on their altitudes and acceptance to install saffron (*Crocus sativus* L.) growing, a mean number of 30 corms (1.5 to 2.5 cm in diameter) were sown in parcel of 9 m^2 in each site (Table 1).

Thus, saffron corms were sown in small areas devoted previously to cultivating subsistence crops. Soil preparation, irrigation and weeding of saffron in experimental parcels are done manually by women who accepted to cultivate saffron. Rainfall annual amount and mean annual temperature during 2014 for the oases of Ain Zaatout, Beni Souik, Djemorah and Maafa and in 2015 for the oases of Branis are mentioned in Table 1, calculated according to www.worldweatheronline.com.

Accordingly, Ain Zaatout known also by the name of Beni Farh, is influenced by the local steppe climate, and throughout the year, there is little rainfall there. However, Beni Souik has a warm Mediterranean climate with dry summers. Branis and Djemorah have a desert climate. In Branis, rain is practically non-existent and in Djemorah

Contact address: Khalila Bengouga, Scientific and Technical Research Center for Arid Regions (CRSTRA) Mohamed Khider University Campus, Biskra, Algeria, tel: +213 778173667; e-mail: leila2000_11@yahoo.fr

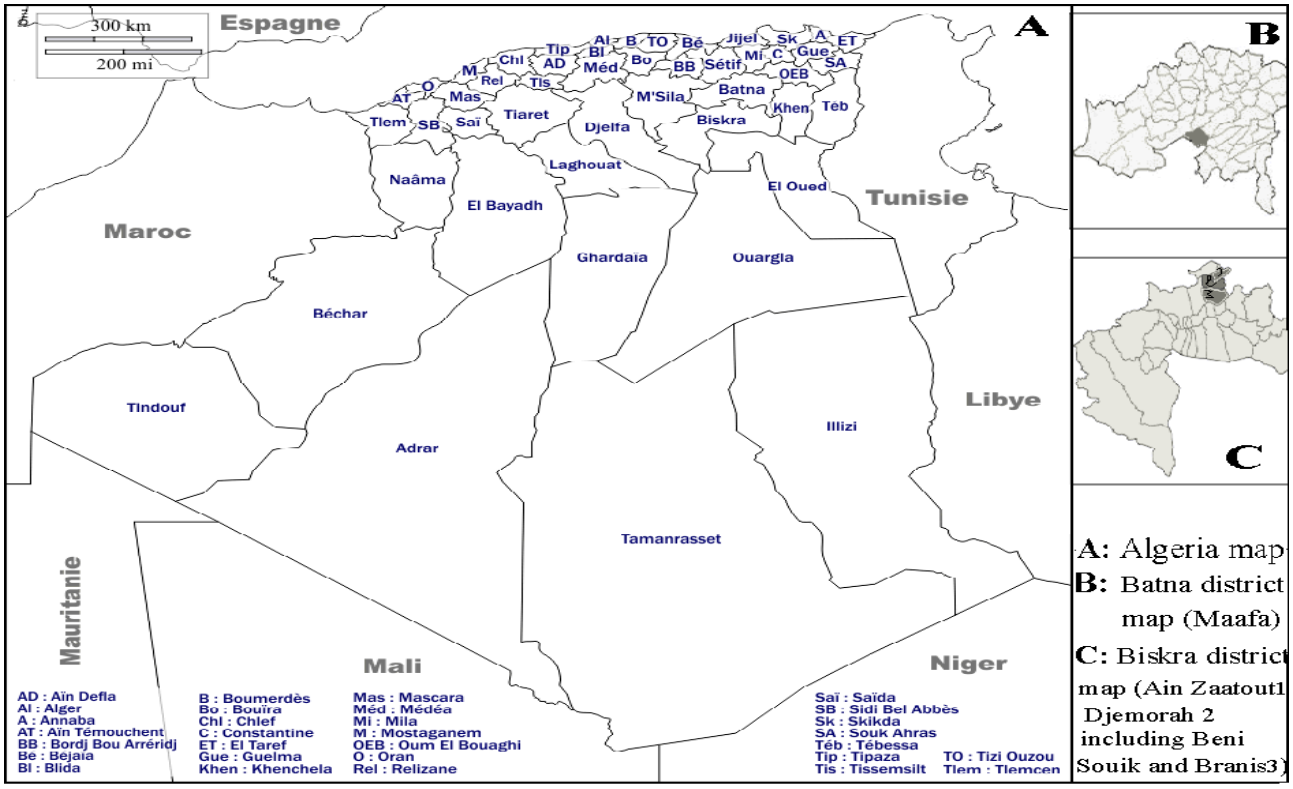

Figure 1 Experimental sites of *Crocus sativus* L. cultivation

Table 1 Altitude, Rainfall, Mean Annual Temperature and sowing dates of different sites

Sites	Sowing date	Altitude (m)	Rainfall (mm)	Temperature (°C)
Ain Zaatout	11/09/2014	831	168.53	22.58
Beni Souik	10/10/2014	553	53.46	21.83
Branis	07/12/ 2014	250	101.2	21.83
Djemorah	09/09/2014	400	53.46	21.83
Maafa	11/09/2014	735.6 (Tasserghinet)	263.81	15

throughout the year, rain is technically non-existent. Meanwhile, Maafa is influenced by the local steppe climate. The rains are light in Maafa and all year round (climate-data.org and planificateur.a-contresens.net).

Results and discussion

Enquiry results

The results in Table 2 show less knowledge of saffron (27%) in the prospected regions. The unfamiliarity of the population with saffron, that it is a plant of moderate countries with arid summer (Mediterranean and steppe climate) originated from the Mediterranean seaside where it grows on mountains of low altitude, is probably due to the fact that previously in North Africa, saffron existed only in scarce indigenous gardens (Chevalier, 1926). It has not been operated especially in arid and semi-arid regions and most interrogated women recognised saffron as a spice and not a plant.

The women did not oppose its cultivation. On the contrary, 59% of them accepted to sow the corms because as rural women of these regions they are interested to improve family income. Especially if they were informed that saffron is the principal source of revenue for the households of mountainous zones in the region of Taliouine-Taznakht in Morocco (Aboudrare, Aw-Hassan and Lybbert, 2014). Also, 55% of inquired women persist in supporting its cultivation even with the exigency in manual work as rural women were informed that saffron needs 95 days of work (flowers collection, prunage) by household. These tasks have to be realised in October-November. Most of them assumed that they will accomplish the work themselves. Meanwhile, Aboudrare, Aw-Hassan and Lybbert (2014) reported the cut of saffron dried leaves is equally assumed by women. The corms harvest, soil work, sowing, fertilisation and irrigation are essentially masculine tasks. Thus, women answered that they will encourage their family members to contribute in the fulfilment of the growing as it is a family work that needs the involvement of adults as well as young people.

Table 2 Knowledge and acceptance of *Crocus sativus* L. cultivation in Algeria (%)

Site	Ain Zaatout	Beni Souik	Maafa	Djemorah	Branis	Total
Ni	24	20	34	2	20	100
KS	16	10	1	0	0	27
AS1	10	18	18	2	11	59
AS 2	8	18	18	2	9	55

Ni – number of women enquired by site; KS – knowledge of saffron; AS1 – acceptation to cultivate saffron for its important income; AS2 – acceptation to cultivate saffron even with its exigency of manual work

Saffron cultivation

Saffron presented an adaptation aptitude in sites of an altitude inferior to 600 masl; and it bloomed in the same year in the case of Beni Souik and Djmorah. Flowering dates are staggered in an increasing gradient in the different sites (Table 3): Beni Souik, Ain Zaatout, Djemorah, Maafa and Branis. The length of the period between the date of sowing and flowering (Fig. 2) depends on sowing precocity and tardiness. Sure enough the oasis of Beni Souik has the last date of sowing which is more tardy than those adapted in Morocco (Ait Oubahou and Eloutman, 2002); even though it registered the shortest duration (35 days); this duration is similar to the reports by Skinner, Parker and Ghalehgolabbehbahani (2017) who reported that for the planted saffron corms in mid-late August or early September in watered soil flowering begins after 30–40 days. Meanwhile, all the flowering stages take place during November; these results are analogous to those reported by Lahmadi et al. (2013) though the authors registered the flowering only during the second year of plantation after a sowing realised on the 3rd October 2010.

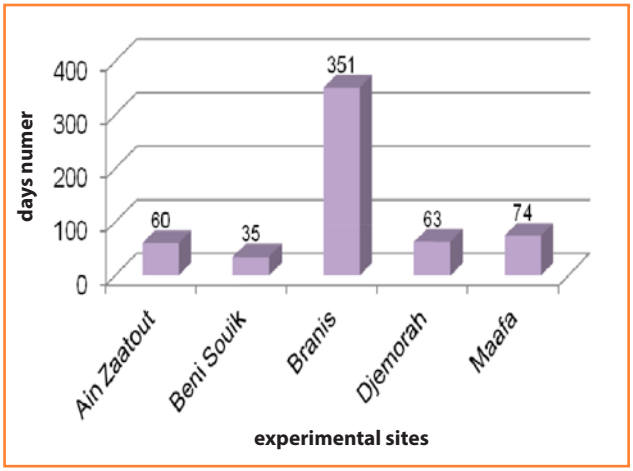

Figure 2 The length of the period between sowing and flowering dates

Flowering precocity depends especially on altitude and temperature factors, knowing that saffron growing needs altitudes between 600 and 1200 m, and the optimal temperature for flowering of about 17° C (Devant, 2008; Molina et al., 2005); thus, the site of Ain Zaatout with the highest altitude (831 m) presented the most precocious date of flowering. And it is probable that site's saffron that has the best quality as altitude is a determining factor of major metabolites (crocin, picrocrocin and safranal) quality with positive effect particularly on crocins content. (Zarinkamar, Tajik and Soleimanpour, 2011; Lage et al., 2009). However, the sites of Beni Souik and Djemorah presented more precocious flowering dates than the site of Maafa even though this site has a higher altitude; this can be attributed to low temperature registered in the oases of Maafa (11 °C) during November 2014 compared to the two oases of Beni Souik and Djemorah which recorded the suitable mean temperature (17 °C) during November 2014 (worldweatheronline.com). Temperature would be the main criterion for estimating the time of flower emergence in this plant. Flower initiation in saffron starts in the late spring; however, flowers appear in the early autumn (Behdani, 2011). Meanwhile, the site of Branis presented the last date of flowering that is due to tardy sowing of corms in this oasis.

Saffron yield obtained in the five oases (Table 3) is low because of the small size of the corms (1.5 to 2.5 cm) given to rural women to be sown; meanwhile, planting larger-sized corms significantly improved spice yield and daughter corm production in the second year (McGimpsey, Douglas and Wallace, 1997); thus, quality of saffron has not been studied in this year. Nevertheless, we estimate that saffron yield in Ain Zaatout (Beni Farh) is the best one that is attributed to the deep drain soil, rich in organic manure

Table 3 Saffron yields in different sites

Site	Flowering date	NC	NF	%
Ain Zaatout	10/11/2014	30	15	50
Beni Souik	14/11/2014	30	2	6.66
Branis	23/11/2015	30	2	6.66
Djemorah	11/11/2014	30	3	10
Maafa	24/11/2014	30	1	3.33
Total	/	150	23	15.33

NC – number of corms, NF – number of flowers

and the satisfactory doses and time of the first irrigation which is the most important factor in initiating the flower emergence in saffron (Behdani, 2011).

Conclusion

The results obtained in the first year of saffron cultivation in mountainous oases of Algeria are promising. *Crocus sativus* L. has presented an adaptation aptitude in sites of altitudes inferior to 600 m (Beni Souik and Djemorah) and a flowering was recorded in the same year of plantation. Indeed, altitude combined with temperature is proved to be a determinant factor of saffron flowering in these sites. As those oases have their special agricultural systems, palm date and arboriculture which must be conserved and since small areas are available to grow up crops, saffron can be introduced as a medicinal plant of high value in association to the ancient agricultural systems to ensure double production of these oases which will increase income of rural population. In order to spread the saffron culture in different Algerian regions, more growers around the country begin to cultivate it; thus, a better appreciation of regional differences effect on saffron life cycle will be obtained to explain the differences of flowering occurrence according to factors such as altitude, temperature, pedo-climatic factors, irrigation and the date of sowing.

References

ABOUDRARE, A. – AW-HASSAN, A. – LYBBERT T.J. 2014. Importance socio-économique du Safran pour les ménages des zones de montagne de la région de Taliouine-Taznakht au Maroc. In Revue Marocaine des Sciences Agronomiques et Vétérinaires, vol. 2, 2014, no. 1, pp. 5–14.

AIT-OUBAHOU, A. – EL-OTMANI, M. 1999. Saffron Cultivation in Morocco. In Saffron. Ed Harwood academic publishers, 1999, pp. 87–94.

AIT OUBAHOU, A. – ELOUTMAN, M. 2002. Fiche technique la culture du safran. In Bulletin mensuel d'information et de liaison du PNTTA, 2002, no. 91, MADREF/DERD, 4 p.

BEHDANI, M. A. 2011. Saffron (*Crocus sativus* L.). In Future Crops, 2002, no. 1, pp. 203–208.

DEVANT, I. 2008. La Culture du safran. Fiche technique. Chambre d'Agriculture d'Indre et Loire, 2008, 4 p.

https://fr.climate-data.org/afrique/algerie-164/consultedon 19/12/2019 and 22/12/2019

https://planificateur.acontresens.net/afrique/algerie/wilaya_de_biskra/beni_souk/2504558.html consulted on 21/12/2019

CHEVALIER, A. 1926. La culture du Safran. Revue de botanique appliquée et d'agriculture coloniale, 6e année. In bulletin, 1926, no. 59, pp. 407–419.

GARCIN, D. G. – CARRAL, S. 2007. Le safran marocain entre tradition et marché. Ed FAO 2007, 73 p.

LAGE, M. – FAIZ, C. – CANTRELL, C.L. 2006. Developmental project for introducing saffron (*Crocus sativus* L.) as an alternative crop in other Moroccan Regions. In Acta horticulturae, 2006, no. 739, pp. 49–52.

LAGE, M. – GABOUN, F. – BAKHY, K. – DAKAK, H. – ZOUAHRI, A. 2009. Sustainable production of high quality saffron (*Crocus sativus* L.) in some Moroccan areas. In Acta horticulturae, 2009, no. 850, pp. 235–238.

LAHMADI, S. – GUESMIA, H. – ZEGUERROU, R. – MAAOUI, M. – BELHAMRA, M. 2013. La culture du safran (*Crocus sativus* L.) En régions arides et semi-arides cas du sud est algérien. In Journal Algérien des Régions Arides, 2013, no. Spécial, pp. 18–27.

McGIMPSEY, J. A. – DOUGLAS, M. H. – WALLACE, A. R. 1997. Evaluation of saffron (*Crocus sativus* L.) production in New Zeland. In New Zeland Journal of Crop and Horticulture Science, vol. 25, 1997, no. 2, pp. 159–168.

MOLINA, R.V. – VALERO, M. – NAVARRO, M. – GUARDIOLA, J.L. 2005. Temperature effects on flower formation in saffron (*Crocus sativus* L.). In Scientia Horticulturae, 2005, no. 103, pp. 361–379.

SCHMIDT, M. – BETTI, G. 2006. Saffron (*Crocus sativus*): An evaluation of the scientific literature. A joint project; Herbresearch Germany and Medicinal and Aromatic Plants R & D. Version of May 18, 2006, 17 p.

SKINNER, M. – PARKER, B.L. – GHALEHGOLABBEHBAHANI, A. 2017. Saffron Production: Life Cycle of Saffron (*Crocus sativus*). University of Vermont, North American Center for Saffron Research and Development, 2017, 2 p.

www.worldweatheronline.com: consulted on 24/12/2019

ZARINKAMAR, F. – TAJIK, S. – SOLEIMANPOUR, S. 2011. Effects of altitude on anatomy and concentration of crocin, picrocrocin and safranal in *Crocus sativus* L. In Australian Journal of Crop Science, vol. 5, 2011, no. 7, pp. 831–838.

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APHIDS (*HEMIPTERA*: *APHIDIDAE*) ON PLUM AND CHERRY PLUM IN BULGARIA

Pavlin VASILEV*, Radoslav ANDREEV, Hristina KUTINKOVA

Agricultural University, Plovdiv, Bulgaria

The species complex and infestations of aphids on plum (*Prunus persica*) and cherry plum (*Prunus cerasifera*) in Bulgaria were investigated during the period 2013–2018. Nine species from the family *Aphididae* were found: *Brachycaudus helichrysi* Kaltenbach (leaf-curling plum aphid), *Hyalopterus pruni* Geoffroy (mealy plum aphid), *Phorodon humuli* Schrank (hop aphid), *Brachycaudus prunicola* Kaltenbach (brown plum aphid), *Brachycaudus cardui* Linnaeus (thistle aphid), *Brachycaudus persicae* Passerini (black peach aphid), *Rhopalosiphum nymphaeae* Linnaeus (waterlily aphid), *Aphis spiraecola* Patch (spiraea aphid) and *Pterochloroides persicae* Cholodkovsky (peach trunk aphid). The dominant species on plum are *Hyalopterus pruni* and *Brachycaudus helichrysi*. The first species is more widespread and of significantly higher density. The dominant species on cherry plum are *Phorodon humuli* and *B. helichrysi*. The species *Brachycaudus prunicola* is widespread both on plum and cherry plum in Bulgaria. It was found only on twigs, and therefore cannot be considered as a dangerous pest on fruit-bearing plum trees. The other species, some of them described as dangerous pests on plum, are today fairly rare and occur in low density, thus posing no danger to orchards.

Keywords: *Brachycaudus*, *Hyalopterus*, *Phorodon*, *Rhopalosiphum*, *Pterochloroides*, *Aphis*

In recent years, the areas with stone fruit orchards in Bulgaria have considerably exceeded the areas with other fruit crops (Agrostatistics, 2016). Plum orchards (including cherry plum) take second place in the country, after cherries, with the area of over 6,700 ha. Plum is attacked by a number of pests and requires a well-organized system of plant protection measures for the protection of fruits, foliage and wood. The most economically important pests are red plum maggot, plum sawfly and some aphids. According to the authors of the Good plant protection practices on plum (Lecheva et al, 2006), dangerous pests from the group of aphids are only three species: plum-thistle aphid *Brachycaudus cardui* L., mealy peach aphid *Hyalopterus amygdali* Blanchard and mealy plum aphid *Hyalopterus pruni* Geoffroy. Seven aphid species on plum in Bulgaria are described by Grigorov, Tashev and Grigorov (2004): leaf-curling plum aphid *Brachycaudus helichrysi* Kalt., *B. cardui*, brown peach aphid *Brachycaudus prunicola* Kaltenbach, *H. pruni*, peach trunk aphid *Pterochloroides persicae* Chol., waterlily aphid *Rhopalosiphum nymphaeae* L. and black peach aphid *Brachycaudus persicae* B.d.F.

Aphids in Bulgaria have not been studied in depth by the end of the last century and the aim of the present study was to establish species composition, distribution and rate of infestation of aphids on plum and cherry plum in our country.

Material and method

Surveys were conducted in plum and cherry plum orchards during 2013–2018: in 2013 – only in the Plovdiv district, in the following two years – in orchards of 128 municipalities across all 28 districts of Bulgaria – in 2014, the southern part of the country, and in 2015 the northern part. The number of the surveyed locations depended on the area of the respective district. Single observations were conducted over the next three years: 2016–2018. In each of the surveyed orchards, a minimum of 200 shoots from 10–20 trees randomly located in the area, were examined. The percentage of infested shoots for each aphid species was estimated. The data were then converted to a five-grade scale similar to the one developed by Mikhailova, Straka and Apostolov (1982), where the grade 0 indicates no infested shoots; grade 1 – less than 5% infested shoots; grade 2 – between 5 and 15% of infested shoots; grade 3 –between 15 and 50% of infested shoots and grade 4 – more than 50% of infested shoots. The maps presented in "Results and Discussion" show only where the aphids are found for the species of minor importance as pests.

When the aphid species could not be identified visually by the coloration of the individuals in a colony or by the type of the damage, microscope slides were prepared according to the method of Martin (1983). The keys of Shaposhnikov (1964), Blackman and Eastop (2000) and Leclant (2000) were used for identification.

Results and discussion

Nine aphid species (*Hemiptera*: *Aphididae*) were found feeding on plum (*Prunus domestica*) and cherry plum (*Prunus cerasifera*) in Bulgaria: leaf-curling plum aphid *Brachycaudus helichrysi* (Kaltenbach), mealy plum aphid *Hyalopterus*

Contact address: Pavlin Vasilev, Agricultural University, Department of Entomology, 12 "Mendeleev" blvd., 4000, Plovdiv, Bulgaria, tel. +359 32 654246, e-mail: pavka89@abv.bg; https://www.au-plovdiv.bg/en/

pruni (Geoffroy), hop aphid *Phorodon humuli* (Schrank), brown plum aphid *Brachycaudus prunicola* (Kaltenbach), thistle aphid *Brachycaudus cardui* (Linnaeus), black peach aphid *Brachycaudus persicae* (Passerini), waterlily aphid *Rhopalosiphum nymphaeae* (Linnaeus), spiraea aphid *Aphis spiraecola* (Patch) and peach trunk aphid *Pterochloroides persicae* (Cholodkovsky).

Leaf-curling plum aphid *B. helichrysi* and Mealy plum aphid *H. pruni* are the dominant species. They were found in most of the surveyed places.

B. helichrysi is a widespread species throughout Bulgaria (Fig. 1). It attacks both plum and cherry plum. In the southern part of the country, we found a stronger attack. In the municipalities of Gotse Delchev, Simitli and Stamboliyski, the infested shoots exceeded 50%. The species was not found only in the municipality of Dimitrovgrad. The infestation in northern Bulgaria was weaker and there was no area where it exceeded 15%. The species was not found in seven of the surveyed municipalities – Antonovo, Brusartsi, Dobrich, Pleven, Ruse, Slivo Pole and Yablanitsa. The aphid causes severe deformations by stopping the growth of attacked shoots and given its widespread prevalence it can be concluded that this is one of the most dangerous pests in these fruit crops although in May it migrated to its secondary hosts.

Hyalopterus pruni is the other widespread species in our country (Fig. 2) attacking plum more strongly, but also cherry plum. The aphid was not found in 18 municipalities, all of them in the southern part of the country. However, in the area where the attack develops it is significant and in 7 of the regions surveyed the infested shoots exceeded 50%.

In North Bulgaria obviously the conditions were more favourable for the spread of this aphid and it was established in all surveyed municipalities. A strong infestation of the shoots was recorded in Misia – 53.6%, Levski and Tutrakan – 60.2% and 65.7%, respectively, and in Rousse and Slivo Pole – over 80%. None of the other aphids, found on plum and cherry plum, have shown such a high rate of infestation.

In southern Bulgaria, distribution of the species is limited. It is not established in five municipalities of the Smolyan destrict, four in the Blagoevgrad district, three in the Kardzhali district, two in Plovdiv and Stara Zagora districts and one in Pazardzhik and Pernik districts. Infestation over 50% was registered only in isolated orchards of the municipalities of Dimitrovgrad and Plovdiv.

Unlike *B. helichrysi*, *H. pruni* does not cause leaf-curling and stop the growth of infested shoots, but stay on trees longer – in late spring and summer. The species formed large colonies and because of its high density causes a premature leaf drop, as well as significant collateral damage with the secreted "honey dew". It belongs to the dangerous pests on these fruit crops.

Phorodon humuli is the third most widespread species in Bulgaria (Fig. 3). It was found in more than a half (80) of the surveyed 143 municipalities. The highest density of the aphid was recorded in the Sadovo municipality – more than 50% infestation on shoots. In the municipalities of Peshtera and Panagurishte, the infested shoots made up 38.4% and 28.4% of the whole, respectively. The species was not found in 62 municipalities – 19 in northern Bulgaria and 43 in southern Bulgaria. This includes the whole districts of Burgas, Rousse and Haskovo. The pest has a preference for

Figure 1 Distribution and infestation of *Brachycaudus helichrysi* on plum and cherry plum in Bulgaria during 2013–2018

Figure 2 Distribution and infestation of *Hyalopterus pruni* on plum and cherry plum in Bulgaria during 2013–2018

Figure 3 Distribution and infestation of *Phorodon humuli* on plum and cherry plum in Bulgaria during 2013–2018

Figure 4 Distribution of *B. prunicola*, *B. cardui*, *B. persicae* and *R. nymphaeae* on plum and cherry plum in Bulgaria during 2013–2018

the cherry plum and attacks only some varieties of plums, but usually it has high population density and can cause premature fall of the leaves of infested shoots, although it does not cause leaf-curling and deformations.

Brachycaudus cardui is a relatively rare species on the territory of the country (Fig. 4). In northern Bulgaria the pest is established only in 12 of the surveyed municipalities. The infestations are weak and usually do not exceed 5%. In the southern part of the country the species has a much wider spread, demonstrating a stronger attack. It was established in 21 municipalities in nine of the districts, and in Smolyan and Chepelare the infestations were highest in the whole country, reaching almost 50%. In the municipalities of Zlatograd, Lucky and Tran, average infestations of about 15% were recorded. The infestations were up to 5% in the rest of the surveyed areas. The aphid was not found in 12 districts of the country – Burgas, Varna, Veliko Tarnovo, Vidin, Dobrich, Pleven, Razgrad, Rousse, Targovishte, Sliven, Haskovo and Yambol. The species causes leaf-curling and deformations, but has no importance as a pest because of its low spread.

It was found out that the black peach aphid *Brachycaudus persicae* infested more plum and cherry plum than peach (Andreev and Vasilev, 2017). In these orchards, the aphid was found in 44 of the surveyed regions – almost equally in the northern and southern parts of the country (Fig. 4). The infestations on the shoots made about 5%, the strongest in the municipalities of Antonovo, Kostinbrod, Omurtag and Chepelare. The species was not found in nine of the districts – Veliko Tarnovo, Vidin, Dobrich, Razgrad, Ruse, Silistra, Stara Zagora and Shumen. This species also causes leaf-curling and deformations, but is of no importance as a pest on plum and cherry plum because of its low level of infestations.

Brachycaudus prunicola is widespread and was found in most of the areas examined, with the exception of the districts in the southeastern part of the country – Burgas, Haskovo, Yambol, Sliven and Kardzhali. The species occurred in the orchards till September. The aphid causes leaf-curling and strong deformations which stop the growth of the infested shoots, but the aphid colonized only offshoots at the base of plum trees. It can be concluded that the species is not a primary pest on plum and cherry plum despite its wide distribution (Fig. 4).

The trees from the genus *Prunus* are primary hosts for the waterlily aphid *R. nymphaeae*. Due to its specific bio-ecological characteristics, the species was found in relatively few places in the country (Fig. 4). In southern Bulgaria, the species was found only in separate gardens and plantations of the Bourgas, Plovdiv and Stara Zagora regions. In southern Bulgaria, the species was found only in separate orchards of the Bourgas, Plovdiv and Stara Zagora districts. The strongest infestation was registered in the municipality of Plovdiv: 23% on the upper shoots of young garden and 36.7% in the offshoots at the base of plum trees. In northern Bulgaria the species was established only in 4 districts – Varna, Gabrovo, Dobrich and Silistra. In the municipalities of Varna and Devnya, the infestations were the strongest – 6.4% and 5.0%, respectively. In all other areas where the species was found, the infestations were under 5%. The pest does not cause leaf-curling and deformations but can cause a delay in the growth of the attacked shoots and premature fall of the leaves and in case of higher infestations could be a dangerous pest for young orchards and nurseries.

Two more species of aphids with low density were found on plum and cherry plum: the spiraea aphid (*A. spiraecola*) and the peach trunk aphid (*P. persicae*). The spiraea aphid was established only on offshoots in the municipality of Plovdiv and Mezdra, and the peach trunk aphid was observed on single trees only in the municipality of Plovdiv.

Conclusions

Nine aphid species (*Hemiptera*: *Aphididae*) were found feeding on plum (*Prunus domestica*) and cherry plum (*Prunus cerasifera*) in Bulgaria during 2013–2018.

Dominant species on plum are the mealy plum aphid (*H. pruni*) and the leaf-curling plum aphid (*B. helichrysi*), the first species being more widespread and significantly higher in density. The aphid does not stop the growth of the infested shoots, but causes retarded development. Because of its high density, the species causes a premature leaf drop as well as significant secondary damage with the secreted "honey dew". The species is a dangerous pest in late spring and summer. *B. helichrysi* causes leaf-curling and strong deformations which stop the growth of the infested shoots. The species is a dangerous pest in early spring.

Dominant species on cherry plum are the hop aphid (*P. humuli*) and (*B. helichrysi*). Both species have high density in spring. *P. humuli* usually has a higher density and can cause premature fall of the leaves of the infested twigs.

The brown plum aphid (*B. prunicola*) is widespread on both plums and cherry plums in the country. The aphid causes leaf-curling and stunted growth of the infested shoots, but the aphid colonized only offshoots at the base of the plum trees, which significantly reduces its importance as a pest.

The rest of the species – *B. cardui*, *B. persicae*, *R. nymphaeae*, *A. spiraecola* and *P. persicae* are found relatively rarely and in low density, because of which they are not a danger to orchards. *B. cardui* and *R. nymphaeae* however, have the potential to rapidly increase their density under certain conditions, and their significance as pests should not be underestimated.

References

AGROSTATISTICS. 2016. Production of fruits – harvest ´2015. Ministry of Agriculture and Food, Sofia. Annual report.

ANDREEV, R. – VASILEV, P. 2017. Aphids (*Hemiptera*: *Aphididae*) on peach trees in Bulgaria. In Agricultural Sciences, vol. 9, 2017, no. 22, pp. 29–36.

BLACKMAN, R. L. – V. F. EASTOP. 2004. Aphids on the World's Crops. An Identification and Information Guide. Chichester, UK : John Wiley, 2004.

GRIGOROV, S. – TASHEV, D. – P. GRIGOROV. 2004. Aphids (Aphidoidea, Homoptera) from Bulgaria and their control. Plovdiv : Academic print at Agricultural University, 2004.

LECLANT, F. 2000. Les Pucerons des plantes cultivees clefs d'identificatin. III – Cultures fruitieres. INRA, Paris, France, 2000.

LECHEVA, I. – PETROV, P. – NAKOVA, M. et al. 2006. Good plant protection practices on stone fruits. Sofia : National Services of Plant Protection, 2006.

MARTIN, J.H. 1983. The identification of common aphid pests of tropical agriculture. In Tropical Pest. Management, 1983, no. 29, pp. 395–411.

MIKHAILOVA, P. – STRAKA,F. – APOSTOLOV, I. 1982. Plant-protection prognosis and signalization. Sofia : Zemizdat, 1982.

SHAPOSHNIKOV, G. 1964. Suborder Aphidodea – Aphids. from Insect identification guide of European part of SSSR. Moscow – Leningrad : Publishing House "Science", 1964, pp. 489–616.

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QUALITY PARAMETERS OF KIWIBERRIES GROWN IN THE CZECH REPUBLIC

Miroslav HORÁK

Mendel University in Brno, Czech Republic

The present study evaluated the quality of kiwiberries produced in the territory of the Czech Republic in 2018 and 2019. *Actinidia arguta* is a very hardy and flexible species which can produce fully ripe fruits even in the setting of moderate climatic zones unlike commercially available *A. chinensis* as a variety which needs a longer season with higher temperatures to become fully ripe. Once harvested, the fruit was studied for soluble solids content, titratable acidity, antioxidant activity and the content of malic, citric and ascorbic acids. Unlike commercially available kiwifruits, kiwiberries lack hair and can be consumed unpeeled. The results of the present study confirmed the notable potential of kiwiberries consisting namely in the levels of antioxidants present in the skin of the fruit. Kiwiberry fruits feature a higher level of acidity than is common in conventional commercial varieties of *A. chinensis* as citric acid is the type of acid prevailing in the fruit at all times.

Keywords: kiwiberry, skin, antioxidant activity, *Actinidia arguta*

Kiwiberry is a member of the same genus as kiwifruit and presents a species of fruit possessing a great potential to expand the segment of food with increased content of biologically active substances on markets around the world. The commercial production of kiwiberry is more recent than that of kiwifruit; it had not started until the 1980s (Debersaques et al., 2015). Currently, New Zealand, USA, Chile, China and Australia are major global producers of kiwiberry along with some of European countries such as France, Belgium or Germany (Latocha, Vereecke and Debersaques, 2018).

A. arguta is among the most widespread species of the *Actinidia* genus. The fruit is smooth and lacks hairs; it weighs 5–10 g and is much smaller than the commercially available kiwifruit. While *A. arguta* is mostly bright green in colour, it may also be pale green, pink up to bright dark red or violet; sometimes, the colour may change during the storage period (Ferguson, Testolin, Huang 2016). The great resistance to frost is typical for kiwiberries when compared with the better known kiwifruit (Drzewiecki et al. 2016). Kiwiberry is richer in taste than kiwifruit; it also features a distinct, aromatic manifestation of tropical fruits. With the positive health effects of the substances contained in the fruit (Rush et al. 2006), kiwiberry is often referred to in the context of superfoods (Ferguson and Ferguson, 2003, Lim, 2012). Wojdyło et al. (2017) report that with the high representation of substances kiwiberry is considered a functional food and ranked as one of the most nutritious fruits globally. For its high levels of phenolic and mineral substances, chlorophylls, carotenoids and ascorbic acid, the fruit is often used in Chinese traditional medicine (Dawes and Keene, 1999; Nishiyama, Fukuda and Oota, 2005).

Material and method

Experimental material

The experimental material comprised of two varieties of *Actinidia arguta*, 'Issai' and 'Ananasnaya,' both grown at a site in Lednice, Czech Republic; as the fruits were ripening in almost identical periods, both varieties were harvested in October decade 3 and 2 in 2018 and 2019, respectively. In both cases, fruits were harvested when they were fully coloured and reached both the size and the degree of hardness corresponding to that variety. The fruits were picked manually and once harvested, they were stored at -28 °C until further processing. For comparison, *Actinidia chinensis* fruits (the varieties of 'Hayward' and 'Soreli') were bought in available commercial shopping chains; these counted 50 fruits in both 2018 and 2019.

Prior to the analysis, the fruits were removed from the freezer and left at a room temperature until they thawed. Subsequently, the skin was removed from each of the fruits and weighed. Each of the samples consisted of 10 fruits in triplicate, i.e. a total of 30 fruits for each of the *A. arguta* varieties. For *Actinidia chinensis*, it involved 10–15 fruits in triplicate, i.e. a total of 30–45 fruits.

Soluble solids

The soluble solids content (SSC) was established using a digital refractometer of PR-32α (Atago, Japan). The fresh fruits were crushed to obtain juice which was subsequently measured. The resulting value was expressed in °Brix degrees.

Contact address: Miroslav Horak, Mendel University in Brno, Faculty of Horticulture, Department of Post-Harvest Technology of Horticultural Products, Valtická 337, 691 44 Lednice Czech republic, Phone: +420 519 367 268, e-mail: miroslav.horak@mendelu.cz

Titratable acidity

To determine titratable acidity (TA), the fresh fruits were homogenised, 5 g were removed from the homogenate, 10 ml of water added to the same and total titratable acidity established through the method of potentiometric titration. The obtained values were recalculated as the citric acid equivalent and then recalculated and specified in g per 100 g of fresh matter.

Organic acids

Each sample was diluted by demineralised water (the 1 : 10 proportion) and subsequently filtered through a micro filter, grain size 0.2 µm. Concentrations of malic, citric and ascorbic acid were determined using an HPLC system with the Chrom SDS 150 pump, thermostat (at 30 °C) and the Thermo-Spectra System UV 6000 LP DAD Detector (Thermo, USA). The used column: Prevail 5 µm Organic Acid 110A HPLC Column 250 × 4.6 mm, mobile phase 25 mM KH_2PO_4, flow: 1 $ml.min^{-1}$. The absorbance of the acids was set at a wavelength of 210 nm. Determining the concentration employed 10-point calibration system using tartaric acid and malic acid standards. Sample volume was 20 µl.

Antioxidant activity

Determination by means of the FRAP (Ferric Reducing Antioxidant Power) method was done in a pH 3.6 acetate buffer (23 mM sodium acetate trihydrate in a solution of 34 $mmol.l^{-1}$ acetic acid). The reaction mixture contained 12 $mmol.l^{-1}$ of $FeCl_3$ solution, 10 $mmol.L^{-1}$ of 2,4,6-tris(2-pyridyl)-s-triazine in 40 $mmol.l^{-1}$ of HCl solution and a buffer in a ratio of 1 : 1 : 10. 2 ml of the reaction mixture were mixed with 25 µl of a diluted sample (5×) with deionized water in a disposable plastic cuvette (10 mm) and the obtained solution was measured using a Helios β spectrophotometer after 10 minutes at a wavelength of 593 nm.

For the DPPH method, 1.9 ml of DPPH (2.2-diphenyl-1-picrylhydrazyl) a radical solution in methanol (0.1 $mmol.l^{-1}$) was mixed with 0.1 ml of 5× diluted sample with deionized water in a disposable plastic cuvette (10 mm). Absorbency at 515 nm was measured after 30 min using the Specord 50 plus (Analytik Jena AG, Germany) spectrophotometer.

The resulting values of antioxidant activity (Trolox equivalents) for both methods were related to the same molar mass of the studied compounds and Trolox to make them mutually comparable.

Statistical analysis

Analysis of variance was performed and means were compared by the Tukey's multiple range test of significant difference (p <0.05). Statistical analyses were performed using the software Statistica 12.

Results and discussion

The results of the two-year observation made it evident that within the respective years the studied parameters exhibit certain deviations; however, given that the deviations were not very significant, both years can be identified as very similar. The size of the fruits is the underlying quality parameter; in this study, it was determined by means of weight. While the average fruit weight did not exceed 7.3 g for *Actinidia arguta*, the weight of *Actinidia chinensis* fruits reached considerably more than 100 g (Table 1). However, as the small size of *Actinidia arguta* fruits is typical for this species, the weight of fruits is essentially no different than values identified by other authors (Zaouay et al., 2012; Wojdyło et al., 2017; White et al., 2005). For both of the *A. arguta* varieties, fruits were smaller in 2018 (by more than 0.7 g). The pulp to skin ratio is higher in the *A. arguta* fruit where the skin presents a higher proportion of weight out of the total fruit than it is with the *A. chinensis* fruit. The detected results match those achieved as a part of another study (Latocha, Łata and Stasiak, 2015).

The highest amount of sugar, or, the highest level of soluble solids, was determined in the 'Issai' fruit in 2019; in the same year, however, the 'Ananasnaya' fruit reached high values, too; as well, a relatively high value was measured for commercially available 'Soreli' fruits (Table 1). In comparison with the commercially available species, *A. arguta* fruits exhibit corresponding soluble solids levels. The same conclusion was reached by authors as a part of other studies (Wojdyło et al., 2017; White et al., 2005; Krupa, Latocha and Liwin´ ska, 2011). If conditions are appropriate, fruits are capable of generating large quantities of sugar even in rather cold settings. Based on the lower soluble solids value and, simultaneously, the higher

Table 1 Fruit, flesh, peel weight, soluble solids and titratable acidity in *Actinidia* fruit

Species	Variety	Year	Weight (g)			SSC (°Brix)	TA (%)
			fruit	flesh	peel		
Actinidia arguta	'Ananasnaya'	2018	6.42^{a}	5.72^{a}	0.65^{a}	11.58^{b}	1.20^{d}
	'Issai'	2018	6.48^{a}	6.08^{a}	0.37^{a}	12.11^{b}	1.22^{d}
	'Ananasnaya'	2019	7.29^{b}	6.27^{a}	0.79^{a}	13.98^{c}	1.03^{c}
	'Issai'	2019	7.18^{b}	6.76^{b}	0.37^{a}	14.60^{d}	1.02^{c}
Actinidia chinensis	'Hayward'	2018	95.00^{d}	88.8^{c}	6.60^{c}	10.18^{a}	0.84^{b}
	'Soreli'	2018	77.52^{c}	70.70^{d}	5.80^{b}	10.01^{a}	0.81^{b}
	'Hayward'	2019	112.3^{e}	102.8^{e}	9.44^{d}	10.09^{a}	0.85^{b}
	'Soreli'	2019	85.43^{d}	78.65^{d}	6.60^{c}	13.36^{c}	0.56^{a}

Different uppercase letters next to values indicate significant differences in columns (p <0.05)

value of titratable acidity in fruits, 2018 can be identified as a less appropriate year for *A. arguta* fruit to ripen. 'Issai' was assessed to contain a relatively high value (1.22%); an almost identical level of 1.2 was identified for 'Ananasnaya' (Table 1). While the larger acidity is typical for *A. arguta* varieties, when years are warmer, they are capable of reducing the level of acidity to below 1% as confirmed for 'Ananasnaya' in 2019. For acidity, citric acid is represented to the largest extent in any of the 4 varieties studied. The fruits also contain malic acid, the proportion of which is considerably lower in the pulp than in the skin with respect to citric acid. This differing proportion was largely observed for the 'Hayward' variety when the pulp contains more than 2.5-fold higher amount of malic acid than that of citric acid while in the skin both of the acids are present in nearly identical concentrations. This tendency was observed for all of the varieties; it was, however, exhibited in none of the samples to such a degree as it was for the variety mentioned above, i.e., 'Hayward' (Table 2). Similar citric acid concentrations in *A. arguta* were measured by other authors as well (Boyes, Strübi and Marsh 1996; Zhou-Li et al. 2013; Latocha, 2017).

Ascorbic acid is another acid of those studied which is significantly involved in the health benefits of kiwi fruit; it is generally considered as quite a good source of the acid as was confirmed through the results of the present work. Overall, it can be stated that a higher ascorbic acid concentration was the one observed in the pulp of any of the studied varieties, which is in conflict with the study (Latocha, Łata and Stasiak, 2015), where an opposite trend was observed, i.e., a higher ascorbic acid concentration in the skin compared with the pulp. Similar ascorbic acid concentrations in the fruit are reported by other authors as well (Latocha, Łata and Stasiak, 2015; Latocha 2017; Latocha et al., 2013; Nishiyama et al., 2004; Cesoniene, Daubaras and Viskelis, 2004). For the present study, the highest value was found for 'Issai' in 2018 with fruits containing 141.4 mg per 100 g of FW (Table 2 and 3). Some smaller levels were found for 'Soreli' in the same year. Out of the studied varieties, 'Ananasnaya' exhibited the lowest ascorbic acid levels in both years of study.

Antioxidant activity was studied using two methods – FRAP and DPPH. The results confirmed that for both *Actinidia chinensis* and *Actinidia arguta*, fruits are a good source of antioxidants compared with other fruit species found in the moderate climate zone. In terms of pulp, the highest values were measured for the variety of 'Soreli' when the FRAP method marked the level of 1.283 and the DPPH technique detected the level of 1.309 (Table 2). While the kiwi fruit skin exhibited a higher concentration of substances with antioxidant effects in any of the cases, at the species level, a significant difference was detected between *A. arguta* and *A. chinensis*. The commercially available varieties of 'Soreli' and 'Hayward' showed to have the antioxidant activity 2–3 times higher in the skin compared with the pulp, and the *A. arguta* skin exhibited more than 15 times the concentration as the one observed for the pulp (Table 2). These results match those achieved by other authors (Latocha et al. 2013; An et al., 2016; Leontowicz et al., 2016).

Table 2 Concentration of organic acids and antioxidant activity in *Actinidia* fruit pulp

Species	Variety	Year	Malic acid (g.100 g FW^{-1})	Citric acid (g.100 g FW^{-1})	Ascorbic acid (mg.100 g FW^{-1})	Antioxidant activity (mmol TE.l^{-1})	
						FRAP	DPPH
Actinidia arguta	'Ananasnaya'	2018	4.34 ±0.68^{e}	8.93 ±0.98^{e}	38.7 ±2.21^{a}	0.64 ±0.20^{d}	0.73 ±0.18^{c}
	'Issai'	2018	4.66 ±0.32^{e}	8.69 ±0.71^{e}	141.4 ±16.3^{d}	0.93 ±0.29^{e}	0.75 ±0.07^{c}
	'Ananasnaya'	2019	3.72 ±0.47^{d}	7.66 ±1.73^{c}	57.6 ±14.2^{b}	0.48 ±0.05^{b}	0.55 ±0.05^{a}
	'Issai'	2019	3.67 ±1.37^{d}	8.02 ±2.09^{d}	72.8 ±18.0^{c}	0.66 ±0.16^{d}	0.64 ±0.13^{b}
Actinidia chinensis	'Hayward'	2018	2.14 ±0.69^{a}	6.82 ±1.43^{ab}	52.0 ±12.6^{b}	0.41 ±0.11^{a}	0.56 ±0.19^{a}
	'Soreli'	2018	3.04 ±0.56^{c}	7.56 ±0.46^{c}	134.0 ±21.5^{d}	1.283 ±0.10^{f}	1.31 ±0.07^{e}
	'Hayward'	2019	2.60 ±0.79^{b}	7.03 ±0.84^{b}	75.1 ±11.9^{c}	0.58 ±0.11^{c}	0.71 ±0.13^{c}
	'Soreli'	2019	1.93 ±0.45^{a}	6.64 ±1.27^{a}	73.0 ±13.6^{c}	0.89 ±0.24^{e}	0.98 ±0.24^{d}

Different uppercase letters next to values indicate significant differences in columns (P <0.05)

Table 3 Concentration of organic acids and antioxidant activity in *Actinidia* fruit skin

Species	Variety	Year	Malic acid (g.100 g FW^{-1})	Citric acid (g.100 g FW^{-1})	Ascorbic acid (mg.100g FW^{-1})	Antioxidant activity (mmol TE.l^{-1})	
						FRAP	DPPH
Actinidia arguta	'Ananasnaya'	2018	3.16 ±0.67^{ab}	5.34 ±0.79^{c}	24.5 ±12.5^{bc}	8.51 ±3.13^{d}	10.33 ±1,43^{e}
	'Issai'	2018	3.83 ±0.92^{b}	6.64 ±0.46^{d}	18.8 ±7.3^{b}	11.27 ±2.28^{e}	14.18 ±1.44^{f}
	'Ananasnaya'	2019	2.76 ±0.64^{a}	4.59 ±0.95^{b}	33.5 ±6.29^{c}	6.18 ±1.34^{d}	7.81 ±1.70^{d}
	'Issai'	2019	3.37 ±0.32^{b}	6.83 ±0.94^{d}	55.0 ±10.3^{d}	10.12 ±1.68^{e}	12.39 ±2.15^{e}
Actinidia chinensis	'Hayward'	2018	4.06 ±0.10^{c}	4.32 ±0.56^{b}	8.6 ±0.7^{a}	1.13 ±0.15^{a}	0.98 ±0.13^{a}
	'Soreli'	2018	4.56 ±0.97^{c}	5.54 ±0.91^{c}	24.2 ±0.7^{b}	3.83 ±0.12^{c}	3.31 ±0.63^{c}
	'Hayward'	2019	3.37 ±0.85^{b}	3.88 ±0.56^{a}	5.2 ±4.3^{a}	1.16 ±0.14^{a}	1.73 ±0.11^{b}
	'Soreli'	2019	2.37 ±0.30^{a}	3.52 ±0.32^{a}	14.0 ±7.4^{ab}	2.32 ±0.18^{b}	3.27 ±0.19^{c}

Conclusion

As a species, *Actinidia arguta* is undoubtedly a very prospective species of kiwi; one that extends the scope of application of the genus as such to the colder regions. Even despite the smaller size, the fruits are tasty and refreshing; they contain a well-balanced proportion of not only sugars and acids, but also substances with antioxidant effects. The smooth skin of the fruits is a great advantage as it is not necessary to be removed prior to eating. It is precisely the high antioxidant potential in the skin which provides a good indicator of the fact that in terms of nutritional composition, *A. arguta* fruits outdo even normally commercially available fruit of *A. chinensis*, more specifically, the 'Hayward' a 'Soreli' varieties. The content of vitamin C, or, ascorbic acid, in kiwi fruits is considerably high; in particular, the 'Issai' variety has a high level in the pulp when the studied fruits contained over 140 mg per 100 g of FW. In terms of soluble solids and titratable acidity of fruits, the two kiwi species seemed to be consistent with each other except that the *A. arguta* fruit is more typical with its slightly higher content of titratable acidity, which is largely evident through citric acid as the prevailing type of acid that reached over 8 g per kg of FW in some of the samples. For a fruit crop grower, *A. arguta* presents, as a species, a definitely attractive alternative to traditional fruit species of colder zones, such as apple, pear, cherry etc. With its notable aromatic manifestation featuring a distinct taste of tropical fruits as well as the well-balanced proportion of sugars and acids, the popularity of the species is sure to rise as evidenced through the increase in scientific studies focusing precisely on the research in the health-beneficial bioactive substances contained in kiwiberry. *A. arguta* could also be a promising species for the processing industry, since it is not necessary to remove the skin from fruits. The disadvantage of these fruits is more difficult harvesting, to determine the exact date of harvest it is necessary to know the physical characteristics of each variety. The gradual ripening of the fruit also complicates the harvest.

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References

AN, X. – LEE, S.G. – KANG, H. – HEO, H.J. – CHO, Y.S. – DO, K. 2016. Antioxidant and anti-inflammatory effects of various cultivars of kiwi berry (*Actinidia arguta*) on lipopolysaccharide-stimulated RAW 264.7 cells. In J. Microbiol. Biotechnol., vol. 26, 2016, no. 8, pp. 1367–1374.

BOYES, S. – STRÜBI, P. – MARSH, H. 1996. Sugar and organic acid analysis of *Actinidia arguta* and rootstock-scion combinations of *Actinidia arguta* Lebensm. In Wiss.Technol., vol. 30, 1996, pp. 390–397.

CESONIENE, L. – DAUBARAS, R. – VISKELIS, P. 2004. Biochemical composition of berries of some kolomicta kiwi (*Actinidia kolomicta*) cultivars and detection of harvest maturity. In Acta Hortic, vol. 663, 2004, pp. 305–308.

DAWES, H.M. – KEENE, J.B. 1999. Phenolic composition of kiwifruit juice. In J. Agric. Food Chem., vol. 47, 1999, pp. 2398–2403.

DEBERSAQUES, F. – MEKERS, O. – DECORTE, J. – VAN LABEKE, M.C. – SCHOEDL-HUMMEL, K. – LATOCHA, P. 2015. Challenges faced by commercial kiwiberry (*Actinidia arguta* Planch.) production. In Acta Hortic., vol. 1096, 2015, pp. 435–442. https://doi.org/10.17660/ActaHortic.2015.1096.52

DRZEWIECKI, J. – LATOCHA, P. – LEONTOWICZ, H. – LEONTOWICZ, M. – PARK, Y.S. – NAJMAN, K. – WEISZ, M. – EZRA, A. – GORINSTEIN, S. 2016. Analytical methods applied to characterization of *Actinidia arguta*, *Actinidia deliciosa*, and *Actinidia eriantha* kiwi fruit cultivars. In Food Anal. Method, vol. 9, 2016, pp.1353–1366.

FERGUSON, A.R. – FERGUSON, L.R. 2003. Are kiwifruit really good for you. In Acta Horit. vol. 610, 2003, pp.132–138.

FERGUSON, A.R. – TESTOLIN, R. – HUANG, A.H. 2016. The kiwifruit genome. Botanical description (Eds.), Series Title: Compendium of Plant Genomes, Publisher : Springer, 2016, pp. 1–14. ISBN 978-3-319-32272-8.

KRUPA, T. – LATOCHA, P. – LIWIŃSKA, A. 2011. Changes of physicochemical quality, phenolics and vitamin C content in hardy kiwifruit (*Actinidia arguta* and its hybrid) during storage. In Scientia Horticulturae, vol. 130, 2011, pp. 410–417.

LATOCHA, P. – WOŁOSIAK, R. – WOROBIEJ, E. – KRUPA, T. 2013. Clonal differences in antioxidant activity and bioactive constituents of hardy kiwifruit (*Actinidia arguta*) and its year-to-year variability. In J. Sci. Food Agric., vol. 93, 2013, pp. 1412–1419.

LATOCHA, P. – ŁATA, B. – STASIAK A. 2015. Phenolics, ascorbate and the antioxidant potential of kiwiberry vs. common kiwifruit: The effect of cultivar and tissue type. In J. Funct. Foods, vol. 19, 2015, pp. 155–163. doi:10.1016/j.jff.2015.09.024

LATOCHA, P. 2017. The nutritional and health benefits of kiwiberry (*Actinidia arguta*) – a review. In Plant Foods Hum. Nutr., vol. 72, pp. 325–334.

LATOCHA, P. – VEREECKE, D. – DEBERSAQUES, F. 2018. Kiwiberry commercial production – what stage are we at? In Acta Hortic., vol. 1218, 2018, pp. 559–564. DOI: 10.17660/ActaHortic.2018.1218.76

LEONTOWICZ, H. – LEONTOWICZ, M. – LATOCHA, P. – JESION, – PARK, Y.S. – KATRICH, E. – BARASCH, D. – NEMIROVSKI, A. – GORINSTEIN, S. 2016. Bioactivity and nutritional properties of hardy kiwi fruit *Actinidia arguta* in comparison with *Actinidia deliciosa* "Hayward" and *Actinidia eriantha* 'Bidan'. In Food Chem., vol. 196, 2016, pp. 281–291.

LIM, T.K. 2012. *Actinidia arguta*. In Edible Medicinal and Non-Medicinal Plants, Fruits (Dordrecht, The Netherlands: Springer), 2012, pp. 5–11.

NISHIYAMA, I. – YAMASHITA, Y. – YAMANAKA, M. – SHIMOHASHI, A. – FUKUDA T. – OOTA, T. 2004. Varietal difference in vitamin C content in the fruit of kiwifruit and other *Actinidia* species. In J. Agric. Food Chem., vol. 52, 2004, pp. 5472–5475.

NISHIYAMA, I. – FUKUDA, T. – OOTA, T. 2005. Genotypic differences in chlorophyll, lutein, and β-carotene contents in the fruits of *Actinidia* species. J. Agric. Food Chem., vol. 53, 2005, pp. 6403–6407.

RUSH, E. – FERGUSON, L.R. – CUMIN, M. – THAKUR, V. – KARUNASINGHE, N. – PLANK, L. 2006. Kiwifruit consumption reduces DNA fragility: a randomized controlled pilot study in volunteers. In Nutr. Res., vol. 26, 2006, pp. 197–201.

WHITE, A. – NIHAL DE SILVA, H. – REQUEJO TAPIA, C. – HARKER, F. R. 2005. Evaluation of softening characteristics of fruit from 14 species of *Actinidia*. In Postharvest Biology and Technology, vol. 35, 2005, pp. 143–151.

WOJDYŁO, A. – NOWICKA, P. – OSZMIAŃSKI, J. – GOLIS, T. 2017. Phytochemical compounds and biological effects of *Actinidia* fruits. In J. Funct. Foods. vol. 30, 2017, pp. 194–202. https://doi.org/10.1016/j.jff.2017.01.018

ZAOUAY, F. – MENA, P. – GARCIA VIGUERA, C. – MARS, M. 2012. Antioxidant activity and physico-chemical properties of Tunisian grown pomegranate (*Punica granatum* L.) cultivars. In Industrial Crops and Products, vol. 40, 2012, pp. 81–89.

ZHOU-LI, Z. – PING MAN, Y. – YAN LAN, X. – CHANG, W.Y. 2013. Ploidy and phenotype variation of a natural *Actinidia arguta* population in the east of Daba Mountain located in a region of Shaanxi. In Scientia Horticulturae, vol. 161, 2013, pp. 259–265. ISSN 0304-4238.

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ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA IN WINE PRODUCTION BY MALDI-TOF MS BIOTYPER

Miroslava KAČÁNIOVÁ[1, 2]*, Simona KUNOVÁ[1], Jozef SABO[1], Eva IVANIŠOVÁ[1], Jana ŽIAROVSKÁ[1], Soňa FELŠÖCIOVÁ[1], Katarína FATRCOVÁ-ŠRAMKOVÁ[1], Margarita TERENTJEVA[3]

[1]Slovak University of Agriculture, Nitra, Slovak Republic
[2]University of Rzeszow, Rzeszow, Poland
[3]Latvia University of Life Sciences and Technologies, Jelgava, Latvia

The aim of this study was to identify lactic acid bacteria (LAB) in grapes, must and wines. A total amount of 90 samples including grape (n = 30), must (no = 30) and wine (no = 30) were collected from vineyards in Slovakia. LAB were used cultured on MRS agar with subsequent confirmation with MALDI-TOF mass spectrometry (Bruker Daltonics). Altogether, 904 isolates were identified. Members of the family Lactobacillaeceae were the most abundant in grape (60%), must (46%) and wine (51%). *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Weissella* genera and 27 species of LAB were isolated from the examined samples. *Leuconostoc mesenteroides* spp. *mesenteroides* was the most abundant species in grape, must and wine.

Keywords: grape, must, wine, lactic acid bacteria, MALDI-TOF MS Biotyper

The wine production is a complex process in which the microorganisms, including lactic acid bacteria (LAB), contribute to the unique sensory characteristics of wine. In general, wine is expected to be an unavailable environment for microbiological growth due to the intrinsic and extrinsic factors of the products: low pH, high concentrations of ethanol and presence of sulfur dioxide (SO_2) (Spano and Massa, 2006). The LAB associated with wine are represented by the phylum Firmicutes, class Bacilli, order Lactobacillales, families Lactobacillaceae and Leuconostocaceae (Garrity, Bell and Lilburn, 2004). The LAB of *Oenococcus*, *Lactobacillus*, *Pediococcus* and *Leuconostoc* genera may establish bacterial growth in wine (Miranda-Castilleja et al., 2016). The recognition of the groups of wine associated microbiota was established by a combination of methods for phylogenetic analysis and allowing defining the particular groups (Makarova et al., 2006). *Oenococcus oeni* is considered to be adapted for growth in the wine environment, hence, it is widely applied for use in commercial MLF starter cultures. Other *Lactobacillus* species have exhibited a capacity to survive in wine as well (Pozo-Bayon et al., 2005). *Lactobacillus plantarum* is another suitable candidate for application in starter cultures. The produced enzymatic complex of *L. plantarum* is specifically attributed to production of β-glucosidase, which could significantly influence the sensory characteristics of wine; thus, it is important in wine production (Mtshali et al., 2009). Description of protein profile with MALDI-TOF (Matrix-assisted laser desorption/ ionization time-of-flight) mass spectrometry is a prospective method for identification of LAB. The results are compatible with those made by molecular methods. Therefore, MALDI-TOF can be considered a fast, accurate and low-cost method for identification of Gram-positive bacteria such as LAB (Rodríguez-Sánchez et al., 2016).

The aim of this study was to identify LAB collected from grape, must and wine with MALDI-TOF MS Biotyper during the technological process.

Material and method

Materials

Samples of grapes (n = 30) were taken gradually during the period of partial ripening of the fruit directly from the vineyards. Samples were put aseptically into polyethylene bags in August 2018 and stored at 8–10 ° C for shipping to the laboratory. Grapes from vineyards of Central Slovakia and the Nitra wine region, and private vineyards were used. Rheinriesling (n = 3), Welschriesling (n = 3), Palava (n = 3), Pinot Blanc (n = 3), and Grüner Veltliner (n = 3) of white varieties and Cabernet Sauvignon (n = 3), Blaufränkisch (n = 3), Blue Portugal (n = 3), Merlot (n = 3) and Pinot Noir (n = 3) of red varieties were sampled. Samples of new wine "must" (n = 30) were collected at the end of August 2018 and in the middle of September 2018 from the same winery as the grapes. Samples (apx. 100 mL) were collected into 200 mL sterile plastic bottles with screw caps and stored

Contact address: Miroslava Kačániová, Slovak University of Agriculture, Faculty of Horticulture and Landscape Engineering, Department of Fruit Sciences, Viticulture and Enology, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovak Republic, Tel.: +421-905 499 166, e-mail: miroslava.kacaniova@gmail.com

at 8–10 °C. An amount of 200 mL of each unfiltered wine (n = 30 from same winery as grape and must) were collected before microfiltration) and stored at 4 °C in the refrigerator.

Microbiological investigations

For microbiological analysis, the grape samples in physiological saline were processed in 24 h after collection. The Man Rogosa Sharpe agar (MRS, Conda, Spain) agar medium for LAB was applied for microbiological testing. The samples were diluted with sterile physiological saline (0.85%) and decimal dilutions were plated out onto MRS agar for incubation at 30 °C for 72 h microaerophilically. After incubation, the isolates were subcultured on Tryptone soya agar 90% (TSA, Basingstoke, UK) with MRS 10% at 30 °C for 24 h. Typical LAB colonies were identified with MALDI-TOF MS Biotyper (Bruker Daltonics, Germany) (Doan et al., 2012).

Mass spectrometry identification of isolates

Qualitative analysis of LAB isolates was performed with MALDI-TOF Mass Spectrometry (Bruker Daltonics, Germany). Criteria for reliable identification were a score of ≥2.0 at species level (Doan et al., 2012).

Statistical analysis

The statistical processing of the data obtained from each evaluation was done with Statgraphics Plus version 5.1 (AV Trading, Umex, Dresden, Germany). For each replication, the mean was calculated and data were log transformed.

Results and discussion

In our study, the LAB counts isolated from grape berries ranged from 2.24 in Welchriesling to 3.33 log cfu.mL^{-1} in Merlot. The LAB counts in must varied from 3.1 in Blue Portugal to 3.24 log cfu.mL^{-1} in Welschriesling. For wine, the LAB counts were reported from 1.17 in Blue Portugal to 2.09 log cfu.mL^{-1} in Palava (Table 1).

Winery environment is expected to be the main source of microorganisms associated with winemaking. Despite the significance of LAB in wine production, the limited number of studies describes their isolation from grapes (Fleet, 2001). The LAB counts were from 0.48 log cfu.mL^{-1} to 2.06 log cfu.mL^{-1} in Cabernet Sauvignon and Blaufränkisch, but negative results were obtained from white grape varieties (Kántor et al., 2015). Previously reported LAB counts for LAB ranged from 3.12 to 3.24 and 1.17 to 2.09 log cfu.mL^{-1} for must and wine, respectively (Kántor et al., 2015). In total, five genera of LAB were identified in the present study (Table 2). *Lactobacillus* spp. was the most abundant (39%) while *Pediococcus* and *Weissella* the least abundant with 7% of distribution for each genera (Table 2).

Obligately homofermentative *L. alimentarius* with 36 isolates was the most abundant *Lactobacillus* (Table 3). The least abundant *Lactobacillus* spp. were *L. coryniformis* and *L. paracasei* with 10 isolates for each species. The predominant LAB were *Leuconostoc mesenteroides* ssp. *mesenteroides* with 251 isolates and *Lactococcus lactis* with 121 isolates. LAB species normally form a part of microbiota of grapes, musts and wines. Different LAB

Table 1 Number of lactic acid bacteria in log cfu.mL^{-1} (mean±SD)

Sample	Grape	Must	Wine
Rheinriesling	2.49±0.16	3.21±0.04	1.25±0.01
Welschriesling	2.24±0.11	3.24±0.02	1.27±0.04
Palava	2.52±0.16	3.20±0.01	2.09±0.03
Pinot Blanc	2.34±0.10	3.12±0.02	1.41±0.20
Grüner Veltliner	2.47±0.05	3.19±0.07	1.28±0.15
Cabernet Sauvignon	2.78±0.03	3.16±0.05	1.29±0.05
Blaufränkisch	2.74±0.15	3.17±0.04	1.27±0.04
Blue Portugal	2.72±0.14	3.10±0.02	1.17±0.07
Merlot	3.33±0.09	3.22±0.06	1.50±0.04
Pinot Noir	2.45±0.20	3.16±0.06	1.18±0.06

Table 2 Number of isolated species from all samples together

Genera	No. of isolates	No. of species	% of isolates	% of species
Lactobacillus	356	21	39.38	77.78
Lactococcus	121	1	13.38	3.70
Leuconostoc	251	1	27.77	3.70
Pediococcus	119	2	13.16	7.41
Weissella	57	2	6.31	7.41
Total	904	27	100	100

species may be present in must and wines, and usually they include heterofermentative cocci of *Leuconostoc* and *Oenococcus*, homofermentative cocci of *Pediococcus* of Lactobacillaceae, homofermentative, facultative, and strict heterofermentative LAB of Lactobacillaceae family (Fugelsang and Edwards, 2007). In wine grapes of Australian vineyards, (Bae et al., 2006) there were detected *Lactococcus* and *Weissella* which is in line with our results.

Lactobacillus acidophilus in different wine samples of Slovak origin ranged from 1 to 105 cfu.mL^{-1} (Kačániová et al., 2012). *Lactobacillus crispatus* was captured by the RTQ PCR with sensitivity ranging from 1 to 105 cfu.mL^{-1} and identification of *Lactobacillus salivarius* by the RTQ PCR method was done. In our study, only *Lactobacillus acidophilus* with 27 isolates was identified. The presence of *Lactobacillus brevis*, *L. casei*, *L. plantarum*, *L. hilgardii* and Lc. *mesenteroides* have been reported in the other studies (Ruiz et al., 2010) which corresponds to our results. A total of 382 isolates from grapes, 372 isolates from must and 150 isolates from wine samples with score higher than 2 were identified (Table 3).

Table 3 Number of LAB isolates from grape, must and wine

Species of microorganisms	Grape	Must	Wine	Total
	No. of isolates			
Lactobacillus acidophilus	5	15	7	27
Lactobacillus alimentarius	10	20	6	36
Lactobacillus amylolyticus	15	0	10	25
Lactobacillus brevis	5	0	10	15
Lactobacillus casei	0	8	4	12
Lactobacillus coryniformis	3	5	2	10
Lactobacillus delbrueckii* ssp. *delbrueckii	5	8	5	18
Lactobacillus fermentum	18	0	0	18
Lactobacillus fructivorans	5	5	2	12
Lactobacillus hilgardii	5	5	2	12
Lactobacillus nageli	15	0	0	15
Lactobacillus oligofermentans	10	8	0	18
Lactobacillus oris	5	8	2	15
Lactobacillus parabuchneri	10	12	3	25
Lactobacillus paracasei	10	0	0	10
Lactobacillus paracasei* ssp. *paracasei	10	6	0	16
Lactobacillus paracasei* ssp. *tolerans	10	5	0	15
Lactobacillus pentosus	15	0	0	15
Lactobacillus plantarum	5	6	5	16
Lactobacillus saerimneri	14	0	0	14
Lactobacillus sakei	12	0	0	12
Lactococcus lactis	35	65	21	121
Leuconostoc mesenteroides* ssp. *mesenteroides	80	126	45	251
Pediococcus acidilactici	20	25	7	52
Pediococcus pentosaceus	20	35	12	67
***Weissella* spp.**	15	10	7	32
Weissella uvarum	25	0	0	25
Total	382	372	150	904

Altogether, 21 species of *Lactobacillus* were found in our study. The positive and negative properties of *Lactobacillus* sp. in wine have been intensively investigated in the recent years (Manes-Lazaro et al., 2009). Studies of wine-associated *Lactobacillus* species are necessary to recognize those responsible for wine spoilage. This fundamental information could be addressed through the identification and enumeration of the LAB at different stages of vinification (Dols-Lafargue, 2018).

In conclusion, the wine-making microbiota is associated with the microorganisms of grapes, must and vinery environment; hence, the changes in the equilibrium of microbiota could alter the acceptance of wine with both the main quality and safety characteristics which may be affected (Capozzi et al., 2017). The microbiological composition of grape impacts the wine quality highlighting the effects of raw material in wine-making (Berbegal et al., 2019).

Conclusions

In our study, ten different varieties (n = 90) of grapes, musts and wines samples were evaluated. The numbers of isolated LAB ranged between the grape, must and wine samples with 904 isolates which were selected for further identification with MALDI-TOF Biotyper. In terms of LAB diversity, three family of LAB were isolated including Lactobacilaceae, Leuconostocaceae and Streptococcacea represented by five different genera and 27 species. The most abundant species in our study were *Lactococcus lactis* and *Leuconostoc mesenteroides* spp. *mesenteroides* in all types of matrixes. MALDI-TOF MS Biotyper was the appropriate method for quick identification of LAB from grape, must and wine.

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References

BERBEGAL, C. – FRAGASSO, M. – RUSSO, P. – BIMBO, F. – GRIECO, F. – SPANO, G. – CAPOZZI, V. Climate Changes and Food Quality: The Potential of Microbial Activities as Mitigating Strategies in the Wine Sector. In Fermentation, vol. 5, 2019, pp. 85.

CAPOZZI, V. – FRAGASSO, M. – ROMANIELLO, R. – BERBEGAL, C. – RUSSO, P. – SPANO, G. Spontaneous Food Fermentations and Potential Risks for Human Health. In Fermentation, vol. 3, 2017, pp. 49.

DOLS-LAFARGUE, M. Polysaccharide Production by Wine Lactic Acid Bacteria: Negative Trait or Potential Advantage? A Review. In Applied Microbiology, vol. 4, 2018, pp. 143.

FLEET, G.H. Wine. In Food Microbiology Fundamentals & Frontiers ed. DOYLE, M.P. – BEUCHAT, L.R. – MONTVILLE, T.J. Washington DC : ASM Press, 2001, pp. 747–772.

FUGELSANG, K.C. – EDWARDS, C.G. Wine Microbiology: Practical Applications and Procedures. 2nd ed. New York, USA : Springer, 2007, 393 p.

GARRITY, G.M. – BELL, J.A. – LILBURN, T.G. Taxonomic outline of the Procaryotes. Bergey's Manual of Systematic Bacteriology. 2nd ed., New York : Springer-Verlag, 2004.

KAČÁNIOVÁ, M. – HLEBA, L. – POCHOP, J. – KADASI-HORAKOVA, M. – FIKSELOVA, M. – ROVNÁ, K. Determination of wine microbiota using classical method, polymerase chain method and Step One Real-Time PCR during fermentation process. In Journal of Environmental Science and Health, Part B, vol. 47, 2012, pp. 571–578.

KÁNTOR, A. – KAČÁNIOVÁ, M. – KLUZ, M. Natural microflora of wine grape berries. In Journal of Microbiology, Biotechnology and Food Science, vol. 4, 2015, no. 1, pp. 32–36.

MAKAROVA, K. – SLESAREV, A. – WOLF, Y. – SOROKINE, A. – MIRKIN, B. – KOONIN, E. – PAVLOV, A. – PAVLOVA, N. – KARAMYCHEV, V. – POLOUCHINE, N. – SHAKHOVA, V. – GRIGORIEV, I. – LOU, Y. – ROHKSAR, D. – LUCAS, S. – HUANG, K. – GOODSTEIN, D.M. – HAWKINS, T. – PLENGVIDHY, V. – WELKER, D. – HUGHES, J. – GOH, Y. – BENSON, A. – BALDWIN, K. – LEE, J.H. – DIAZ-MUNIZ, I. – DOSTI, B. – SMEIANOV, V. – WECHTER, W. – BARABOTE, R. – LORCA, G. – ALTERMANN, E. – BARRANGOU, R. – GANESAN, B. – XIEF, Y. – RAWSTHORNE, H. – TAMIR, D. – PARKER, C. – BREIDT, F. – BROADBENT, J. – HUTKINS, R. – O'SULLIVAN, D. – STEELE, J. – UNLU, G. – SAIER, M. – KLAENHAMMER, T. – RICHARDSON, P. – KOZYAVKIN, S. – WEIMER, B. – MILLS D. Comparative genomics of lactic acid bacteria. Proceedings of the National Academy of Sciences of the United States of America, vol. 42, 2006, pp. 15611–15616.

MANES-LAZARO, R. – FERRER, S. – ROSSELLO-MORA2AND, R. – PARDO, I. *Lactobacillus oeni* sp. nov., from wine. In International Journal of Systematic Evolutionary Microbiology, vol. 59, 2009, pp. 2010–2014.

MIRANDA-CASTILLEJA, D.E. – MARTINEZ-PENICHE, R.A. – ALDRETE-TAPIA, J.A. – SOTO-MUNOZ, L. – ITURRIAGA, M.H. – PACHECO-AGUILAR, J.R. – ARVIZU-MEDRANO, S.M. Distribution of native lactic Acid Bacteria in Wineries of Queretaro, Mexico and Their Resistance to Wine-Like Conditions. In Frontiers in Microbiology, vol. 7, 2016, pp. 1769.

MTSHALI, P.S. – DIVOL, B. – VAN RENSBURG, P. – DU TOIT, M. Genetic screening of wine-related enzymes in *Lactobacillus* species isolated from South African wines. In Journal of Applied Microbiology, vol. 108, 2009, no. 4, pp. 1389–1397.

POZO-BAYON, M.A. – G-ALEGRIA, E. – POLO, M.C. – TENORIO, C. – MARTÍN-ÁLVAREZ, P.J. – CALVO DE LA BANDA, M.T. – RUIZ-LARREA, F. – MORENO ARRIBAS, M.V. Wine volatile and amino acid composition after malolactic fermentation: Effect of *Oenococcus oeni* and *Lactobacillus plantarum* starter cultures. In Journal of Agriculture and Food Chemistry, vol. 53, 2005, pp. 8729–8735.

RODRÍGUEZ-SÁNCHEZ, B. – ALCALÁ, L. – MARÍN, M. – RUÍZ, A. – ALONSO, E. – BOUZA, E. Evaluation of MALDI-TOF MS (Matrix-Assisted Laser Desorption-Ionization Time-of-Flight Mass Spectrometry) for routine identification of anaerobic bacteria. In Anaerobe, vol. 42, 2016, pp. 101–107.

RUIZ, P. – IZQUIERDO, P.M. – SESE'NA, S. – PALOP, M.L. Analysis of lactic acid bacteria populations during spontaneous malolactic fermentation of Tempranillo wines at five wineries during two consecutive vintages. In Food Control, vol. 21, 2010, no. 1, pp. 70–75.

SPANO, G. – MASSA, S. Environmental stress response in lactic acid bacteria: beyond *Bacillus subtillis*. In Critical Review in Microbiology, vol. 32, 2006, pp. 77–86.

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COMPARATIVE TECHNOLOGICAL CHARACTERISTIC OF THE ALIGOTE 61-6 AND ALIGOTE N 10 CLONES, CULTIVATED IN THE SOIL AND CLIMATIC CONDITIONS OF THE REGION OF PLEVEN

Tatyana YONCHEVA*, Zdravko NAKOV

Institute of Viticulture and Enology, Pleven, Bulgaria

In the period 2011–2013 a technological characteristic of the Ukrainian clone Aligote 61-6 was made at the Institute of Viticulture and Enology – Pleven. The Bulgarian candidate-clone Aligote N 10 was used for control. During the grapes ripening, the dynamics of sugar accumulation was monitored. Upon technological maturity the indicators of the yields were accounted and mechanical analysis was performed. The chemical composition of the must, the obtained wines and their organoleptic qualities were analyzed. In its mechanical composition, Aligote 61-6 was typically wine one and it did not differ significantly in the texture and structure of the cluster and berry from the control. The theoretical yield of both clones was high. They exhibited good sugar accumulation and similar acid content. Grapes from the control had better technological indicators for obtaining wines of optimal chemical composition and quality. In the 2011 and 2013 vintages, the control wines exceeded those of the Ukrainian clone in terms of sugar-free extract content. The experimental wines Aligote N 10 had higher titratable acidity compared to the Ukrainian clone. The difference in the phenolic substances ratio and the colour intensity in the samples from both clones were insignificant. The control wines were superior in their organoleptic qualities to those of the Ukrainian clone.

Keywords: Aligote, grapes, wine, chemical composition, organoleptic characteristics

In the study of the grapevine species and varieties, it had been important to apply objective and accurate methods of ampelographic characterization. Its aim was to clarify their origin and distribution, to show their basic botanical features, their agrobiological specifics and their economic and technological qualities (Roychev, 2012).

Much of the widespread industrial vine varieties had been characterized by considerable interspecific diversity. That was due to mutational variability and the occurrence of morphological and physiological changes transmitted to the offspring and sometimes impairing the economic qualities of the varieties and their economic efficiency (Katerov et al., 1990). Worldwide, the clone selection has been the most common method for extending the structure of vineyards of varieties within *Vitis vinifera*. It aims to improve the individual agro-biological, technological and economic characteristics of a variety. As a result, diversification of the vine collection is achieved, based on the selection of clones with high levels of realization of the potential economic productivity and quality indicators of grapes and wine (Petrov et al., 2009; Meneghetti et al., 2010).

Aligote is an old French variety, originating from the Burgundy region, but common in other European wine-growing countries. In Bulgaria it has been grown mainly in the northeastern part of the country, on an area of about 263 ha, representing 1.28% of the white wine varieties and 0.472% of all vineyards. Aligote is a medium ripening wine variety. The grapes mature in the first half to mid-September. The vines are distinguished for medium growth, high fertility and yields. It is grown on stem training with mixed pruning. The variety is not resistant to diseases and pests, it is resistant to low winter temperatures but not to drought. The most suitable for its cultivation are carbonate black earth and gray-brown forest soils. In France, through clonal selection, 8 clones of Aligote variety were created, out of which 263, 264 and 651 were of medium yield, and 402, 591, 920, 935 and 936 of high yield (Roychev, 2012).

By its mechanical composition, Aligote cluster is typically a wine variety with a high theoretical yield of the must. It shows its valuable technological qualities in the moderately cool regions of northeastern Bulgaria, where the grapes have optimal sugar accumulation of 18–21% and titratable acids 7 $g.l^{-1}$. In warmer regions, with a higher temperature sum, the variety demonstrates rapid sugar accumulation (up to 22–24%) and reduced titratable acidity (5–5.8 $g.l^{-1}$). Quality white dry wines and sparkling wines are produced by Aligote (Radulov, Babrikov and Georgiev, 1992; Roychev, 2012).

The factors determining the wine features are of a complex nature. The biochemical indicators of the grapes and must related to the variety, the applied agricultural and technical practices, the conditions in the process of vinification, etc. are of importance. The influence of terroir – the mineral composition of the soil and the climatic conditions (sunshine, temperature, precipitation) in the cultivation area – is also of significance. In Romania, Aligote is one of the most common wine varieties. Bora et al. (2016) studied Aligote wines composition, 2015 vintage, and found

Contact address: Tatyana Yoncheva, Institute of Viticulture and Enology, 5800 Pleven, Bulgaria, 1"Kala tepe"str., e-mail: t_ion@abv.bg

a low ratio of malic acid, mineral components (potassium, calcium, iron) and high glycerol content. Aroma is one of the most important indicators of wine features. Vararu et al. (2015) identified 38 components of the aromatic profile of Romanian Aligote wines. Of the analyzed terpenes, norisoprenoids, aldehydes, ketones, higher alcohols, benzene derivatives, fatty acids and esters, the highest amounts found were of 3-methyl-1-butanol, decanoic acid, ethyl octanoate and ethyl decanoate.

The objective of this study was to make a technological characteristic of Aligote clone 61-6 and Aligote clone N 10, grown under the soil and climatic conditions of the town of Pleven (central northern Bulgaria).

Material and method

The technological characteristics study of Aligote 61-6 and Aligote N 10 clones was carried out at the Institute of Viticulture and Enology (IVE) – Pleven during the period 2011–2013, and it comprised three consecutive vintages. Aligote 61-6 was a Ukrainian clone (Fig. 2) while Aligote N 10 was a Bulgarian candidate-clone (Fig. 1) used for control.

The studied clones were grown at the Experimental Base of IVE at Ombrella training and planting distance of 3.00/1.30 m. The applied growing practice to the vines was mixed pruning and equal loading – 32 winter eyes per vine (6 spurs of 2 eyes and 2 fruit canes of 10 eyes). During the grapes ripening, the dynamics of sugar accumulation was monitored by a refractometer to determine the grapes' technological maturity. Upon reaching the technological maturity, the productivity indicators were accounted, and a mechanical and chemical analysis of the grapes was performed (Katerov et al., 1990).

The grapes were processed in the Experimental Winery of IVE according to the classical technology for dry white wine making under the conditions of micro-vinification (Yankov, 1992) – crushing, destemming, pressing, sulphuring (50 mg.l^{-1} SO_2), must clarification, adding pure culture dry wine yeast *Saccharomyces cerevisiae Vitilevure* B + C (20 g.hl^{-1}), fermentation temperature 20 °C, racking, further sulphuring, storage.

The grape must chemical composition was determined according to the following methods (Ivanov et al., 1979): sugars, g.l^{-1} – areometer of Dujardin; glucose, g.l^{-1} – iodometric method; fructose, g.l^{-1} – calculation method; titratable acids (TA), g.l^{-1} – titration with NaOH; tartaric and malic acid, g.l^{-1} – method of Pochinok; pH – pH-meter; glucoacidometric index (GAI) – calculation method as the ratio of sugars (%) and TA (g.l^{-1}).

The main indicators of wines chemical composition were analyzed by conventional methods in the wine-making practice (Ivanov et al., 1979; Chobanova, 2007): sugars, g.l^{-1} – Schoorl's method; alcohol, vol. % – distillation method, Gibertini apparatus with densitometry of the distillate density; total extract (TE), g.l^{-1} – Gibertini apparatus with densitometry, density of alcohol-free sample; sugar-free extract (SFE), g.l^{-1} – calculation method (the difference between TE and sugars); titratable acids (TA), g.l^{-1} – titration with NaOH; volatile acids (VA), g.l^{-1} – distillation method with titration with NaOH; tartaric and malic acid, g.l^{-1} – method of Pochinok; total phenolic compounds (TPC), g.l^{-1} – method of Singleton et Rossi; colour intensity, [abs. units] – method of Glories, by measuring the absorption at λ 420 nm; pH – pH-meter. The value of each analyzed indicator of the composition of the experimental wines was average of the measurement of two parallel samples. If a significant difference was found in the values, a third sample was measured and the two closest values were taken into account.

The organoleptic features of the experimental samples were determined according to 100-score scale for the indicators: colour, aroma, taste and general impression (Tsvetanov, 2001) by a nine-member tasting committee. The tasting score of the experimental wines was average value of the committee members' estimates, eliminating the highest and the lowest ones.

Figure 1 Aligote clone N 10

Figure 2 Aligote clone 61-6

Results and discussion

At technological maturity of the studied clones, the productivity indicators were accounted, and a mechanical analysis of the grapes was performed (Table 1).

The mechanical analysis showed that Aligote 61-6 was typically a wine clone and there was no significant difference in the texture and structure of the cluster and the berry from the control (Aligote N 10). On the average for the period of the study the weight per cluster of Aligote 61-6 was 201.3 g and that of Aligote N 10 was 221.5 g. Aligote 61-6 cluster had a higher rachis content – 3.89% and fewer berries – 96.11%, compared to the control – 3.40% rachis and 96.60% berries. The average mass per 100 berries from the Ukrainian clone was insignificantly higher (202.67 g) than the control (193.33 g), which also determined the difference in their berry structure. Aligote 61-6 berry had less skins (10.03%) and seeds (3.85%) and higher mesocarp content – 86.12%. As for Aligote N 10 its berry had more skins (10.66%) and seeds (4.05%) and less mesocarp – 85.29%. The theoretical yield of the Ukrainian clone was 82.77% and of the control – 82.39%. The insignificantly higher theoretical yield of Aligote 61-6 was the result of the higher rate of mesocarp in the berry.

During the grape ripening of the studied clones, the intensity of sugar accumulation was investigated (Fig. 3 a, b, c).

In 2011, the reporting of the sugar accumulation dynamics in both clones started on 30/08/, as the grapes from Aligote N 10 having 15.9% sugars and from Aligote 61-6 – 16.0%. In the following two measurements, it was found that in both clones the accumulation of sugars proceeded at the same rate: from 30/08/ to 06/09/ – by 1.2% each and from 06/09/ to 13/09/ – by 1.4% each. During the period from 13/09/ to 20/09/ the intensity of sugar accumulation decreased by 1.0% for Aligote N 10 and 1.2% for Aligote 61-6, due to the decrease in temperatures and the rainfall (Fig. 3a). The grape must analysis revealed that on 20/09/ the sugar content of Aligote N 10 was 19.5% and the titratable acids were 6.30 g.l^{-1}. In Aligote 61-6, the sugars were 19.8% and the titratable acidity lower – 5.93 g.l^{-1}.

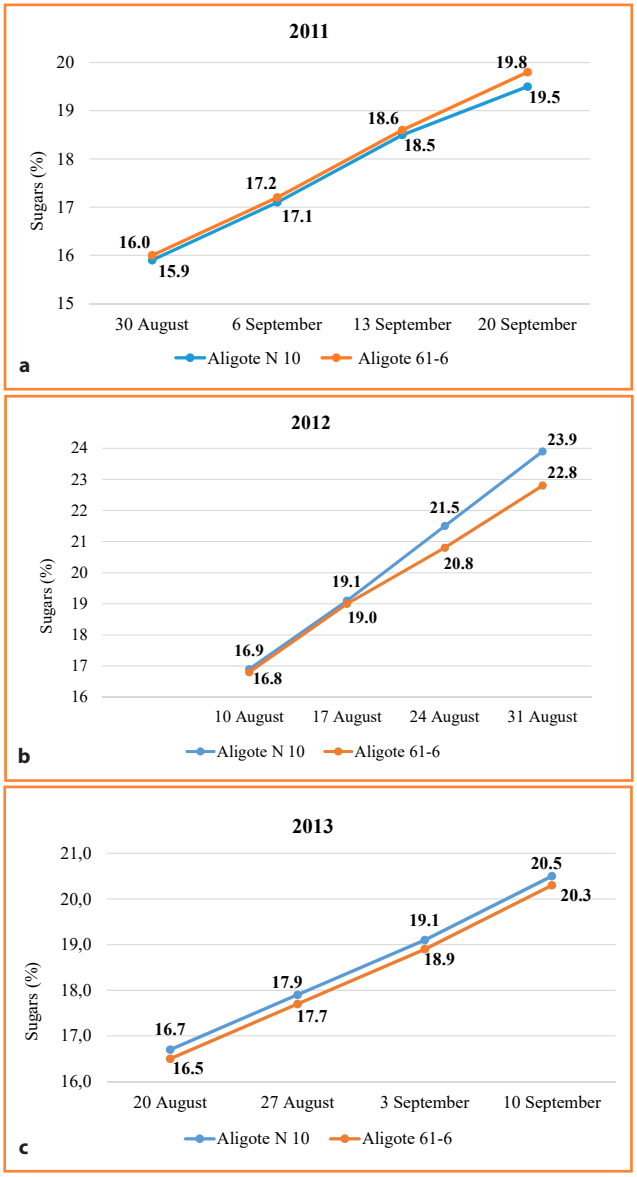

Figure 3 Changes in sugars during the grapes ripening period of the studied Aligote clones

Table 1 Mechanical analysis of grapes from the studied Aligote clones for the period 2011–2013

Vintage	Average mass of a cluster (g)	Cluster texture		Average mass per 100 berries (g)	Berry structure			Theoretical yield (%)
		rachis (%)	berry (%)		skins (%)	seeds (%)	mesocarp (%)	
Aligote N 10								
2011	237.0	2.99	97.01	190.00	9.58	3.79	86.63	84.04
2012	202.0	2.97	97.03	220.00	11.36	4.04	84.60	82.09
2013	225.5	4.24	95.76	190.00	11.05	4.31	84.64	81.05
Average	221.5	3.40	96.60	193.33	10.66	4.05	85.29	82.39
Aligote 61-6								
2011	218.5	3.62	96.38	193.30	9,31	3.52	87.17	84.01
2012	198.0	3.53	96.47	225.00	10,12	4.02	85.86	82.83
2013	187.5	4.53	95.47	190.00	10,67	4.00	85.33	81.46
Average	201.3	3.89	96.11	202.67	10,03	3.85	86.12	82.77

Due to the hot and dry months of July and August 2012, the grapes ripening began considerably earlier than in the preceding year. On 10/08/ the sugar content of Aligote N 10 grapes was 16.9% and that of Aligote 61-6 – 16.8%. That year, the sugar accumulation in the grapes of clone N 10 occurred more intensively: from 10/08/ to 17/08/ by 2.2%, from 17/08/ to 24/09/ and from 24/08/ to 31/08/ by 2.4%. In the grapes of clone 61-6 the sugar accumulation was from 10/08/ to 17/08/ by 2.2%, from 17/08/ to 24/09/ by 1.8%, and from 24/08/ to 31/08/ by 2.0% (Fig. 3b). The grape must analysis showed that the clones had high sugar ratio – 23.9% (Aligote N 10) and 22.8% (Aligote 61-6) and lower titratable acids – 5.33 g.l^{-1} and 5.85 g.l^{-1}, respectively.

In 2013, the first evaluation was made on 20/08/, where it was found that in the grapes from Aligote N 10 the sugars were 16.7% and in Aligote 61-6 – 16.5%. In the following measurements, no difference in the sugar accumulation intensity was found between both clones. During the periods from 20/08/ to 27/08/ and from 27/08/ to 03/09/ the amount of sugars increased by 1.2% and from 03/09/ to 10/09/ by 1.4% (Fig. 3c). In analyzing the must on 10/09/ the sugar ratio in the grapes was good – 20.5% (Aligote N 10) and 20.3% (Aligote 61-6).

The grapes from both studied Aligote clones were harvested upon reaching technological maturity. The data on the must composition are presented in Table 2. During the study period, both clones showed good sugar accumulation. The average rates were 213.00 g.l^{-1} (Aligote N 10) and 209.67 g.l^{-1} (Aligote 61-6) respectively, as in 2011 and 2013 vintages of both clones had similar sugar content and, in the harvest 2012, the control exceeded significantly the Ukrainian clone 61-6. Of the monosaccharides identified in grape must, fructose was predominant in both clones – 77.58 g.l^{-1} glucose and 125.42 g.l^{-1} fructose (average) for N 10 and 84.58 g.l^{-1} glucose and 125.09 g.l^{-1} fructose (average) for 61-6. An exception was observed only in the must of Aligote N 10, vintage 2011, where their rates were almost equal.

The clones had the specific titratable acidity for Aligote variety. For the studied period, similar acid content was analyzed in the musts of both clones – the average of 5.78 g.l^{-1} (N 10) and 5.93 g.l^{-1} (61-6), as in the 2012 and 2013 vintages of the Ukrainian clone contained more acids compared to the control. The tartaric and malic acids were prevailing from the organic acids in the wine. When analyzing their amount in the samples, no unidirectionality was found in the results. In 2011, the malic acid in Aligote N 10 must predominated, while the tartaric acid was prevailing in 61-6 must. In 2012 vintage, the malic acid predominated in both clones and the tartaric acid in the 2013 harvest. GAI had a higher average rate in the control grapes (average 3.73) than in 61-6 grapes (average 3.54). That indicated that the must from Aligote N 10 had better technological indicators for the production of wines with optimal chemical composition and quality. In both studied clones, the 2012 grapes had a higher content of sugars, GAI rates and lower titratable acidity due to the more favourable weather conditions during the period of grapes ripening phase.

The chemical composition of the experimental wines obtained from the studied clones of Aligote variety is presented in Table 3 (a, b).

The alcohol content in the wines from both clones was on the average 12.87 vol. % (N 10) and 12.67 vol. % (61-6). In the control, the rates were within the range from 12.25 to 13.70 vol. % and in the Ukrainian clone from 12.02 to 13.67 vol. %. The alcohol in the samples corresponded to the sugars in the grapes per vintages and respectively, the highest rates were found in the wines from the 2012 harvest. The alcoholic fermentation in all the test samples occurred normally without deviation and with complete degradation of the fermentable sugars. That was confirmed by the residual sugars in the obtained wines – on the average 1.62 g.l^{-1} (Aligote N 10) and 1.61 g.l^{-1} (Aligote 61-6).

The sugar-free extract ratio was an important indicator of wine composition. Its rates were within the typical range for Aligote variety. In the samples of N 10 the quantity of SFE was in the narrow range from 17.04 to 17.66 g.l^{-1}, while in the samples of 61-6 the difference between the individual vintages was greater – from 16.16 to 17.82 g.l^{-1}. In the 2011 and 2013 harvests, the control wines exceeded those of the Ukrainian clone. In 2012, just the opposite was observed (Table 3a).

The experimental samples had normal titratable and volatile acidity (Table 3b). During the study period, Aligote N 10 wines contained more titratable acids (the average of 6.05 g.l^{-1}) compared to those from the Ukrainian clone

Table 2 Chemical composition of grape must from the studied Aligote clones, for the period 2011–2013

Vintage	Date of harvest	Sugars (g.l^{-1})	Glucose (g.l^{-1})	Fructose (g.l^{-1})	Titratable acidity (g.l^{-1})	Tartaric acid (g.l^{-1})	Malic acid (g.l^{-1})	GAI	pH
Aligote N 10									
2011	20/09/	195.00	100.10	94.90	6.30	3.37	5.29	3.10	3.29
2012	31/08/	239.00	80.10	158.90	5.33	1.12	4.23	4.48	3.27
2013	10/09/	205.00	52.53	122.47	5.70	5.62	3.22	3.60	3.24
Average		213.00	77.58	125.42	5.78	3.37	4.25	3.73	3.27
Aligote 61-6									
2011	20/09/	198.00	88.40	109.60	5.93	5.10	4.22	3.34	3.25
2012	31/08/	228.00	80.10	147.90	5.85	3.97	5.59	3.90	3.26
2013	10/09/	203.00	85.23	117.77	6.00	5.70	2.98	3.38	3.24
Average		209.67	84.58	125.09	5.93	4.92	4.26	3.54	3.25

Table 3a Chemical composition of wines from the studied Aligote clones, in the period 2011–2013

Vintage	Alcohol (vol. %)	Sugars ($g.l^{-1}$)	Total extract ($g.l^{-1}$)	SFE ($g.l^{-1}$)	TPC ($g.l^{-1}$)	Colour intensity (I) [abs. units]
Aligote N 10						
2011	12.25	1.14	18.80	17.66	0.51	0.004
2012	13.70	2.46	19.50	17.04	0.75	0.004
2013	12.65	1.27	18.60	17.33	0.57	0.101
Average	12.87	1.62	18.97	17.34	0.61	0.036
Aligote 61-6						
2011	12.02	1.04	17.20	16.16	0.50	0.006
2012	13.67	2.18	20.00	17.82	0.70	0.008
2013	12.33	1.61	18.70	17.09	0.33	0.090
Average	12.67	1.61	18.63	17.02	0.51	0.035

Table 3b Chemical composition of wines from the studied Aligote clones, in the period 2011–2013

Vintage	Titratable acidity ($g.l^{-1}$)	Tartaric acid ($g.l^{-1}$)	Malic acid ($g.l^{-1}$)	Volatile acidity ($g.l^{-1}$)	pH
Aligote N 10					
2011	6.00	1.84	3.02	0.42	3.12
2012	5.45	1.52	4.86	0.66	3.23
2013	6.70	2.25	3.62	0.54	3.10
Average	6.05	1.87	3.83	0.54	3.15
Aligote 61-6					
2011	5.75	2.21	2.98	0.54	3.05
2012	5.38	1.84	3.25	0.66	3.18
2013	6.00	2.70	2.91	0.60	3.06
Average	5.71	2.25	3.05	0.60	3.10

(the average of 5.71 $g.l^{-1}$). The lowest titratable acidity had the samples from both clones – 2012 vintage. From the organic acids analyzed in all experimental wines there was found a predominance of malic acid over tartaric acid.

The phenolic substances ratio and the colour intensity in the experimental samples were also in the range characteristic of white wines, with minor differences between the wines of the studied clone and the control. The TPC ratio was on the average 0.61 $g.l^{-1}$ (N 10) and 0.51 $g.l^{-1}$ (61-6), with higher rates reported for 2012 vintage for both clones (Table 3a).

The results of the chemical and organoleptic analysis did not show a direct correlation between the content of the studied indicators and the tasting score of the samples (Table 3 (a, b), Fig. 4).

From Aligote N 10 wines, the best organoleptic qualities had the sample of 2011 vintage (77.78 points), where the lowest alcohol and TPC and the highest SFE rates were analyzed. From Aligote 61-6 wines, the best characteristics had the sample from the 2013 vintage (75.50 points), where the lowest TPC rates and the highest titratable acidity were found. During the study period, the control samples surpassed those of the Ukrainian clone in their organoleptic properties (Fig. 4, Fig. 5). The average tasting score of N 10 wines was 77.32 points, and of 61-6 wines it was 75.12 points (Fig. 4).

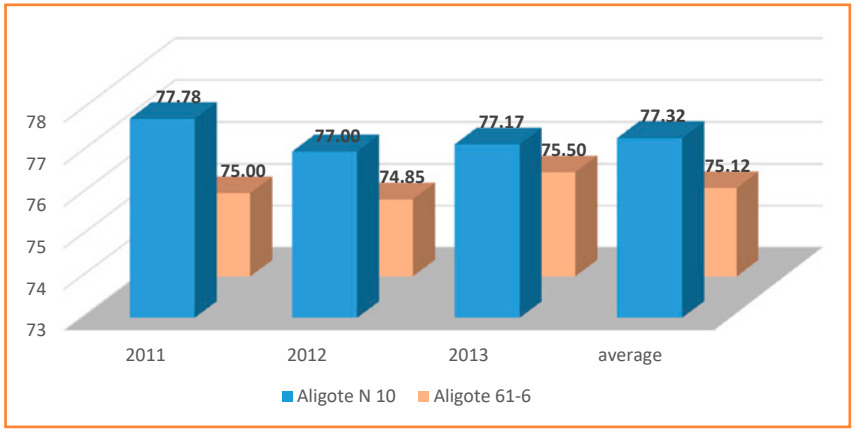

Figure 4 Tasting score of wines from the studied Aligote clones, in the period 2011–2013

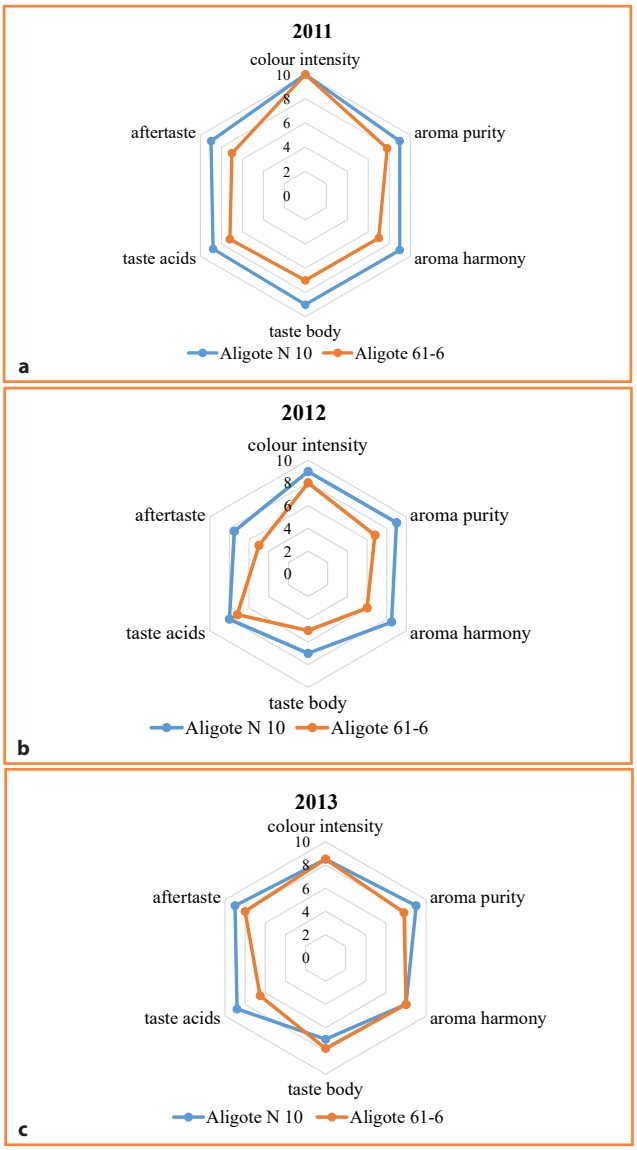

Figure 5 Organoleptic profile of wines from the studied Aligote clones, in the period 2011–2013

That indicated that the control samples had better tasting characteristics in terms of aromatic and taste indicators, harmony and balance (Fig. 5).

Conclusion

On the basis of the obtained results from the comparative technological study, the following could be summarised:

- In its mechanical composition, Aligote 61-6 was typically wine one and it did not differ significantly in the texture and structure of the cluster and berry from the control. Its cluster had a higher rate of rachis (3.89%) and less berries (96.11%) compared to Aligote N 10. Aligote 61-6 berry has less skins (10.03%) and seeds (3.85%) and higher rate of mesocarp – 86.12%. The theoretical yield of both clones was high – 82.77%(Aligote 61-6) and 82.39%(Aligote N 10).
- Both clones showed good sugar accumulation with average rate of 213.00 g.l^{-1} (Aligote N 10) and 209.67 g.l^{-1} (Aligote 61-6) respectively, and similar acid content – the average of 5.78 g.l^{-1} (N 10) and 5.93 g.l^{-1} (61-6).
- In 2011 and 2013 vintages, the control wines were superior to those of the Ukrainian clone in terms of sugar-free extract ratio.
- The experimental Aligote N 10 wines had a higher titratable acidity (the average of 6.05 g.l^{-1}) compared to the Ukrainian clone (the average of 5.71 g.l^{-1}). The samples from the 2012 vintage had the lowest titratable acids. A predominance of malic acid over tartaric acid was observed.
- The difference in the phenolic substance ratio and colour intensity in the experimental samples of the studied clone and the control were insignificant. Higher TPC rates were reported in 2012 vintage wines for both clones.
- No direct correlation was found between the content of the tested chemical indicators and the tasting score. The wines from the control surpassed in organoleptic qualities those from the Ukrainian clone.

References

BORA, F. – DONICI, A. – OŞLOBANU, A. – FIŢIU, A. – BABEŞ, A. – BUNEA, C. 2016. Qualitative assessment of the white wine varieties grown in Dealu Bujorului vineyards, Romania. In Notulae Botanicae Horti Agrobotanici Cluj-Napoca, vol. 44, 2016, no. 2, pp. 593–602. ISSN 0255-965X, 1842-4309. Available at: http://www.notulaebotanicae.ro

CHOBANOVA, D. 2007. Textbook for exercises in enology. Plovdiv : Academic Publishing House of University of Food Technology, 2007, pp. 51–74. ISBN 978-954-24-0082-0.

IVANOV, T. – GEROV, S. – YANKOV, A. – BAMBALOV, G. – TONCHEV, T. – NACHKOV, D. – MARINOV, M. 1979. Practicum in wine technology. Plovdiv : Publishing "Hristo G. Danov", 1979, 531 p.

KATEROV, K. – DONCHEV, A. – KONDAREV, M. – KURTEV, P. – TSANKOV, B. – ZANKOV, Z. – GETOV, G. – TSAKOV, D. 1990. Clonal and sanitary selection. In Bulgarian Ampelography, Sofia : Bulgarian Academy of Science Publishing House, 1990, pp. 195–199.

MENEGHETTI, S. – COSTACURTA, A. – FRARE, E. – CRESPAN, M. 2010. Evaluation of the intra-varietal variability for the clones identification. In Rivista di Viticoltura e di Enologia, vol. 63, 2010, no. 1/4, pp. 93–106. ISSN 0370-7865.

PETROV, V. – NUDYGA, T. – TALASH, A. – GUGUCHKINA, T. – DAUROVA, E. – CHIGRIK, B. – GRYUNER, M. 2009. The formation of the sortment of grapes for high-quality winemaking. In Collection of the International scientific-practical conference "Scientific and applied aspects of the development of viticulture and winemaking at the present stage", All-Russian Research Institute of Viticulture and Enology "Y. I. Potapenko", Novocherkassk, Russia, 2009, pp. 94–100.

RADULOV, L. – BABRIKOV, D. – GEORGIEV, S. 1992. Ampelography with bases of winemaking. Sofia : Zemizdat, 1992, 186 p.

ROYCHEV, V. 2012. Ampelography. Plovdiv : Academic Edition of the Agricultural University, 2012, 576 p. ISBN 978-954-517-146-8.

TSVETANOV, O. 2001. How to taste wine. Sofia : Gourmet, 2001, 94 p. ISBN 954-90809-1-9.

VARARU, F. – MORENO-GARCIA, J. – MORENO, J. – NICULAUA, M. – NEVHITA, B. – ZAMFIR, C. – COLIBABA, C. – DUMITRU, G. D. – COTEA, V. 2015. Minor volatile compounds profiles of Aligote wines fermented with different yeast strains. In Notulae Scientia Biologicae, vol. 7, 2015, no. 1, pp. 123–128. ISSN 2067-3205, 2067-3264. Available at: http://www.notulaebiologicae.ro

YANKOV, A. 1992. Wine making technology. Sofia : Zemizdat, 1992, 355 p.

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EXPRESSION ACTIVITY OF ALLERGEN CODING GENES IN GRAPE VARIETIES USED FOR TOKAJ WINE PRODUCTION

Jana ŽIAROVSKÁ*, Lucia ZAMIEŠKOVÁ, Miroslava KAČÁNIOVÁ

Slovak University of Agriculture in Nitra, Slovak Republic

Vitis vinifera L. is adapted to a very variable range of climates but it mostly grows in the temperate regions of continental Europe. In the Slovak Republic, the Tokaj wine region is one of the territories, where wine production is concentrated. Grape is a popular fruit and when processed, it is used as musts, juices or wine. Some people may suffer from allergic reactions to grapes. Up to now, endochitinases, lipid-transfer protein, and thaumatin were identified as grape allergens. In this study, expression of chitinase and thaumatin allergen was analysed in the grapes of Tokaj region varieties – Hachat Lovelin, Tokay and Muskat Blanc. Expression changes were calculated by the delta delta Ct method. Expression differences of chitinase were found to be similar in these varieties. Thaumatin was found to be variable in its transcription.

Keywords: Tokaj region grape varieties, chitinase, thaumatin, expression

Vitis vinifera L., is one of the oldest cultivated plants all over the world. It is well adapted to a wide range of climates, but it grows especially well from the temperate Mediterranean regions to the continental areas in Central Europe. Grape fruit is widely consumed either directly or as wine. Western Europe is the world's biggest producer of grapes, mainly France, Italy and Spain are the major producers of wines (Pastorello et al., 2003). The health beneficial effects of grapes and wine are very well known due to their high nutritional value and unique phytochemical composition. *Vitis vinifera* is a major source of polyphenols, flavonoids, anthocyanins, phenolic acids, stilbenes, vitamins (A and C), minerals (phosphorus, calcium) and carbohydrates (Arora et al., 2016). It has recently been observed that moderate consumption of grapes or red wine has many health beneficial effects: anti-asthmatic, cardio protective, cytotoxic, anti-aging, hepatoprotective, anti-inflammatory, and antioxidant (Ahmad and Khan, 2012; Masani et al., 2012).

Many of grapes' biochemical and technological characteristics are based on climatic conditions where the plants grow. In this study, grape varieties from the Tokaj wine region were used. The Tokaj wine region is quite a unique one with the following characteristics. It consists of clay or loess soil on volcanic subsoil with sunny microclimate. Some specific grape varieties such as Tokay and Hachat Lovelin have been cultivated in the region for centuries and, together with Muskat Blanc are the only grape varieties that are planted in this region.

Despite many health protective and beneficial effects of *Vitis vinifera*, some people suffer from allergic reactions to this fruit. Major allergens of wine and grapes: 30-kd endochitinase 4A and 4B, 9-kd lipid-transfer protein (LTP) and 24-kd thaumatin were characterized and identified by Pastorello et al. (2003). The precise molecular mechanism of action of grape allergens has not been yet studied and therefore requires our attention and also the need to develop novel diagnostics methods and improve treatment management in this field. Chitinases are the most active protein components in causing wine turbidity (Falconer et al., 2010; Marangon et al., 2011). Chitinases derived from grapes are present in different isoforms (Marangon et al., 2011; Gazzola et al., 2012) and are tolerant to low pH in juice and wine as well as resistant to photolytic enzymes, as most of the pathogenic related proteins (Ferreira et al., 2001; Waters et al., 2005; Van Sluyter et al., 2015). Thaumatin was identified in tropical plant *Thaumatococcus danielii* Benth as a protein having sweet taste. A specific domain common to osmotin-like proteins and a kinase receptor of PR5-like proteins was described in its structure and grouped together as the base of the thaumatin-like protein family (Wang et al., 2011). These proteins are much diversified in their functions and were described to be involved in stress responses (Yan et al., 2017). Thaumatin-like protein structure was defined in grapes by Marangon et al. (2014) and physico-chemical parameters relevant for the haze formation mechanism were determined by these authors.

Beside the relevance in winemaking, thaumatin is defined as a minor grape allergen together with chitinases (Vassilopoulou et al., 2007). Its relevance is important, because grapes are consumed not only processed, but fresh, too, and some severe allergic reactions were reported in the case of grape consumption in the Mediterranean region before (Kalogeromitros et al., 2006). Grape allergy is

Contact address: Jana Žiarovská, Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Department of Genetics and Plant Breeding, Slovak Republic, e-mail: jana.ziarovska@uniag.sk

clinically manifested by severe symptoms mainly in patients suffering from multiple allergies and preferentially to LTP (lipid transfer proteins) containing foods (Vassilopoulou et al., 2007) and an association with peach and cherry allergy was observed (Pastorello et al., 2003). Actually, there is no specific information about the differences on transcriptomic level for the allergens and their genes. In this study, expression of chitinase and thaumatin-like allergen was analysed in the grapes of three grape varieties planted in the Slovak Tokaj wine production.

Material and method

Biological material

Grapes of three varieties of *Vitis vinifera*, L. that are used for producing Tokaj wines were obtained in the season 2018 in the Slovak Tokaj wine region. Only grapes without any infection marks were used for further analysis. Fresh matured grapes of varieties Lipovina (syn. Hachat Lovelin, Lindenblütrige, Hárselevelű, Kerekes, Kereklevelű), Furmint (syn. Tokay, Mosler, Luttenberger, Weisslauber, Edler weisser Tokayer, Malnik, Furmint bianco, Bihari boros, Demjén, Formont, Furmin) and Muškát žltý (syn. Muškát Lunel, White Frontigan, Muskat Blanc, Muscat de Lunel, Muscat de Frontignan, Bárzsing, Fehér muskotály, Gelber Muskateller, Muskateller, Weisse Muskattraube, Muskatel menudo bianco, Moscato bianco) were harvested and after surface cleaning transported immediately to the laboratory where they were kept in -50 °C until further processing.

RNA extraction and cDNA synthesis

The total RNA was extracted using the GeneJet Plant RNA Purification Mini Kit (ThermoFisher) following the manufacturer´s instruction with a modification of the weight of homogenized tissue use. The obtained RNA concentration and A260/A280 nm ratios were determined by Implen Nanophotometer and the integrity of the RNA was checked in 1% agarose gels. cDNA synthesis was performed from 30 ng of total RNA using the Tetro cDNA synthesis kit (BIOLINE) with the oligodT primer.

Analysis of the expression of chitinase and thaumatin

A two-step protocol was used for both, chitinase and thaumatin expression analysis where the gene for actin (GenBank accession AY847627) was used as the internal control during qPCR. Amplification was performed using EliZyme Green MIX AddROX (Elizabeth Pharmacon) in Stratagene Mx3005P thermal cycler (Agilent). The following program was used for chitinase transcripts amplification: 95 °C for 2 minutes followed by 40 cycles of 95 °C for 10 seconds, 60 °C for 40 seconds, ended by dissociation curves analysis of amplified thaumatin products by heating the amplicon from 75 °C to the 95 °C. All the reactions were performed in triplicates. The thaumatin transcript analysis followed the PCR conditions of Žiarovská et al. (2019). Chitinase specific primers were designed on the basis of its sequence stored in the GeneBank under acession DQ267094 and thaumatin specific primers were designed on the basis of coding region of the genomic sequence stored in the GeneBank under accession AF227324.

Results and discussion

Expression profiles of chitinase and thaumatin were analysed in three Tokaj grape varieties – Hachat Lovelin, Tokay and Muskat Blanc. Actin was used as an internal control for the correction of sample-to-sample variation. The generated Cts of actin amplicons ranged from 18.30 (Hachat Lovelin) up to the 20.85 (Tokay). Dissociation curves of amplified actin products calculated during the melting procedure showed a single melting peak with melting temperature (Tm) of 81.8 °C.

Dissociation curves of amplified chitinase products showed a single melting peak with melting temperature (Tm) of 85.8 °C, indicating specific product. Control reactions of NTC (non-template control) generated clearly differentiated products. Cts of chitinase generated amplicons during the real-time PCR analysis varied from 29.70 (Hachat Lovelin) to 31.64 (Tokay). Expression changes of chitinase allergen were calculated by the delta delta Ct method (Livak and Schmittgen, 2001). Chitinase expression was found to be similar in the three analysed grape varieties. The Tokay grape variety was found as having the lowest activity in chitinase transcription activity, counted in 36 fold percentage decreasing when compared to the Hachat Lovelin variety and 63 fold percentage decreasing when compared to the Muscat Blanc variety. Muscat Blanc has the expression level of chitinase very similar when compared to the Hachat Lovelin with the increasing 0.21 fold change (Fig. 1).

Dissociation curves of amplified thaumatin products in the Slovak Tokay grape varieties calculated during the melting procedure showed a single melting peak with melting temperature (Tm) of 87.5 °C, indicating specific product. Control reactions of NTC generated clearly differentiated products. Thaumatin expression was found to be more variable in the three analysed grape varieties when compared to the expression of chitinase. Here again, the Tokay grape variety was found as to have the lowest activity in thaumatin transcription activity, counted in 100 fold percentage decreasing when compared to the Hachat Lovelin variety and 186 fold percentage decreasing when compared to the Muscat Blanc variety. Interestingly, Hachat Lovelin has the expression level of thaumatin 10 times higher when compared to the Muscat Blanc (Fig. 2).

Up to now, only a few studies have existed that analyse the expression activity of grape allergens. Thaumatin expression analysis was performed previously by Žiarovská et al. (2019) in grapes of four red varieties of *Vitis vinifera*, L. that were obtained in the season 2017 in the Sabo winery that belongs to the Malokarpatský wine region. Dornfelder was found to have the lowest activity in thaumatin-like gene activity, mainly when compared to Cabernet Sauvignon and Frankovka modrá. Alibernet, on the other side, has the expression level of thaumatin very similar when compared to Cabernet Sauvignon and Frankovka modrá.

Expression profiles of different thaumatin-like proteins were analysed by Yan et al. (2017) in the varieties Red Globe, Shang-24, Hunan-1 and Shuangyou under the inoculation of three different pathogens with the conclusion that the expression of this gene family is broadly influenced by *Botrytis cinerea*, *Elsinoe necator* and *Elsinoe ampelina*.

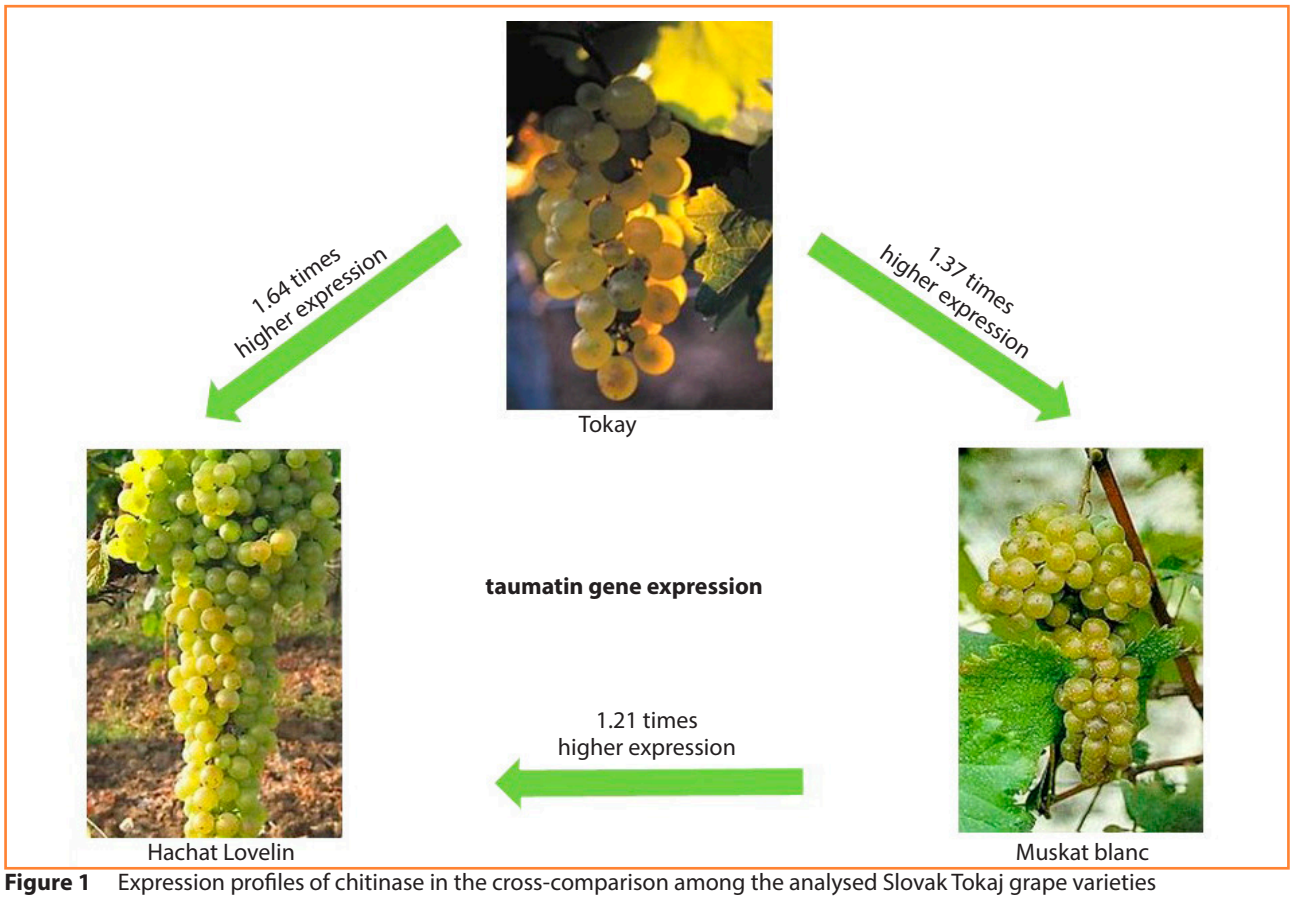

Figure 1 Expression profiles of chitinase in the cross-comparison among the analysed Slovak Tokaj grape varieties

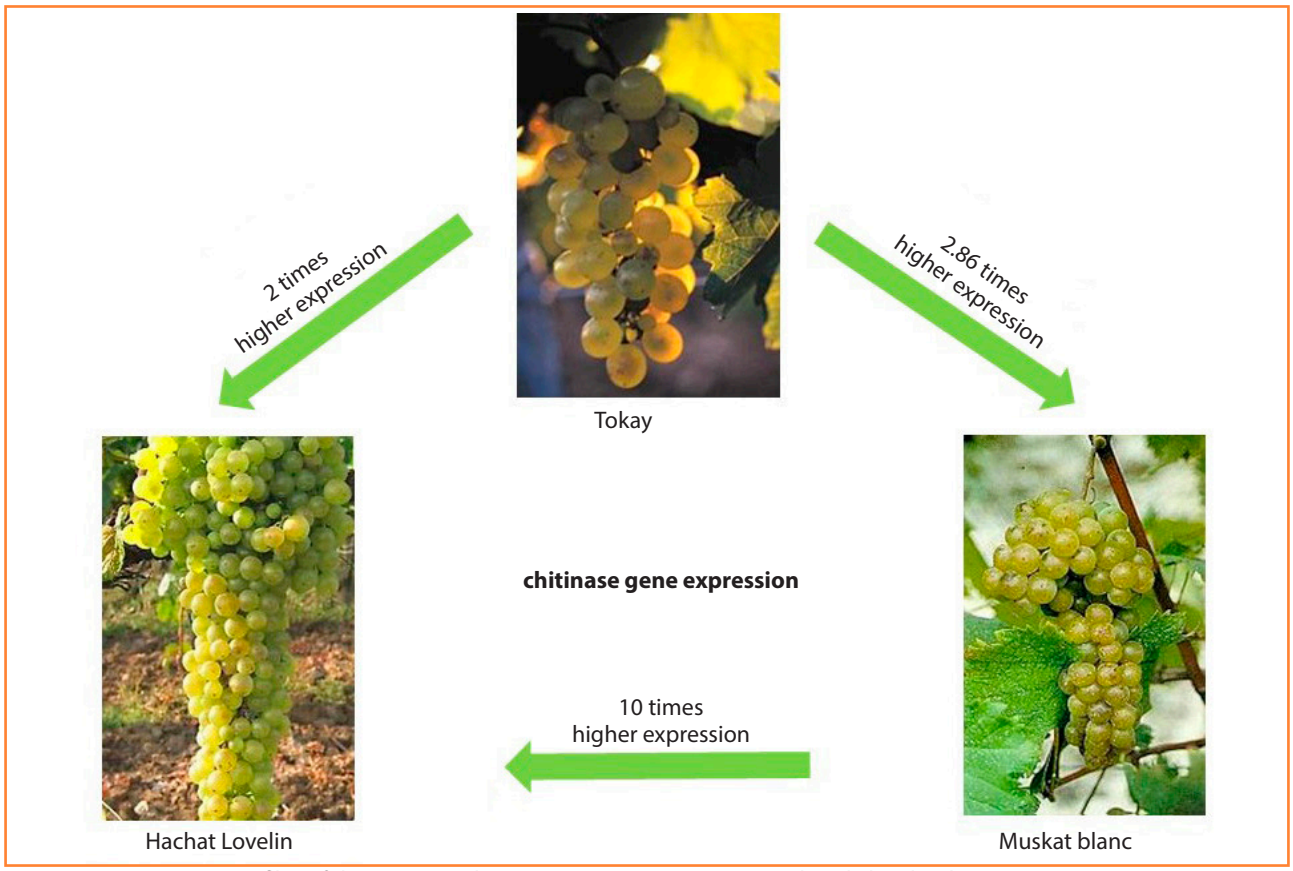

Figure 2 Expression profiles of thaumatin in the cross-comparison among analysed Slovak Tokaj grape varieties

Expression of thaumatin in grape is reported up to now to be affected by different panthogens. Subsequence of inoculation by anthracnose, powdery mildew and *Botrytis* was analysed in the sense of thaumatin-like genes expression in three different grape varieties by Yan et al. (2017) with the conclusion that different genes were increased in their expression following each of the inoculation pattern.

Conclusion

Chitinase and thaumatin are both minor allergens in grapes, but their persistence to wine and juice products is relevant to people suffering from grape allergy. Expression differences of chitinase and thaumatin in the Tokaj region grape varieties – Hachat Lovelin, Tokay and Muskat Blanc show that chitinase is similar in its expression in these varieties, but taumatin is much more variable in transcription. Muscat Blanc was found to have the highest level of thaumatin expression with the expression fold change of 10.

References

AHMAD, F. – KHAN, G. M. 2012. Study of aging and hepatoprotective activity of *Vitis vinifera* L. seeds in albino rats. In Asian Pacific Journal of Tropical Biomedicine, vol. 2, 2012, no. 3, pp. 1770–1774. https://doi.org/10.1016/S2221-1691(12)60492-4

ARORA, P. – ANSARI, S. H. – NAJMI, A. K. – ANJUM, V. – AHMAD, S. 2016. Investigation of anthi-asthmatic potential of dried fruits of *Vitis vinifera* L. in animal model of bronchial asthma. In Allergy, Asthma and Clinical Immunology, vol. 12, 2016, p. 42. https://doi.org/10.1186/s13223-016-0145-x

FALCONER, R.J. – MARANGON, M. – VAN SLUYTER, S.C. – NEILSON, K.A. – CHAN, C. – WATERS, E.J. 2010. Thermal stability of thaumatin-like protein, chitinase, and invertase isolated from Sauvignon Blanc and Semillon juice and their role in haze formation in wine. In Journal of Agriculture and Food Chemistry, vol. 58, 2010, p. 975.

FERREIRA, R.B. – PICARRA-PEREIRA, M.A. – MONTEIRO, S. – LOUREIRO, V.B. – TEIXEIRA, A.R. 2001. The wine proteins. In Trends in Food Science and Technology, vol. 12, 2001, p. 230.

GAZZOLA, D. – VAN SLUYTER, S.C. – CURIONI, A. – WATERS, E.J. – MARANGON, M. 2012. Roles of proteins, polysaccharides, and phenolis in haze formation in white wine via reconstitution experiments. In Journal of Agriculture and Food Chemistry, vol. 60, 2012, p. 10666.

KALOGEROMITROS, D. C. – MAKRIS, M. P. – GREGORIOU, S. G. – KATOULIS, A. C. – STRAURIANEAS, N. G. 2006. Sensitization to other foods in subjects with reported allergy to grapes. In Allergy and Asthma Proceedings, vol. 27, 2006, no. 1, pp. 68–71. https://doi.org/10.2500/aap.2006.27.2882

LIVAK, K.J. – SCHMITTGEN, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. In Methods, vol. 25, 2001, p. 402.

MARANGON, M. – VAN SLUYTER, S.C. – NEILSON, K.A. – CHAN, C. – HAYNES, P.A. – WATERS, E.J. – FALCONER, R.J. 2011. Roles of grape thaumatin-like protein and chitinase in white wine haze formation. In Journal of Agriculture and Food Chemistry, vol. 59, 2011, p. 733.

MARANGON, M. – VAN SLUYTER, S. C. – WATERS, E. J. – MENZ, R. I. 2014. Structure of Haze Forming Proteins in White Wines: *Vitis vinifera* Thaumatin-Like Proteins. In PLOS. https://doi.org/10.1371/journal.pone.0113757

MASANI, Y. A. – MATHEW, N. – CHAKRABORTY, M. – KAMATH, J. V. 2012. Effects of *Vitis vinifera* against Trition-X-100 induced hyperlipidaemia in rats. In International Research Journal of Pharmacology, vol. 3, 2012, no. 12, pp.101–103.

PASTORELLO, E. A. – FARIOLI, L. – PRAVETTONI, V. – ORTOLANI, C. – FORTUNATO, D. – GIUFFRIDA, M. G. – PERONO GAROFFO, L. – CALAMARI, A.M. – BRENNA, O. – CONTI, A. 2003. Identification of grape and wine allergenes as an endochitinase 4, a lipid-transfer protein, and a thaumatin. In Journal of Allergy and Clinical Immunology, vol. 111, 2003, no. 2, pp. 350–359.

VAN SLUYTER, S.C. – McRAE, J.M. – FALCONER, R.J. – SMITH, P.A. – BACIC, A. – WATERS, E.J. – MARANGON, M. 2015. Wine protein haze: mechanisms of formation and advances in prevention. In Journal of Agriculture and Food Chemistry, vol.63, 2015, p. 4020.

VASSILOPOULOU, E. – ZUIDMEER, L. – AKKERDAAS, J. – TASSIOS, I. – RIGBY, N. R. – MILLS, E. N. – van REE, R. – SAXONI-PAPAGEORGIOU, P. – PAPADOPOULOS, N. G. 2007. Severe immediate allergic reactions to grapes: part of a lipid transfer protein-associated clinical syndrome. In International Archives of Allergy and Immunology, vol. 143, 2007, no. 2, pp. 92–102. https://doi.org/10.1159/000098657

WANG, Q. – LI, F. – ZHANG, X. – ZHANG, Y. – HOU, Y. – ZHANG, S. – WU, Z. 2011. Purification and characterization of a CkTLP protein from *Cynanchum komarovii* seeds that confers antifungal activity. In PLoS One, vol. 22, 2011, no. 6, e16930. https://doi.org/10.1371/journal.pone.0016930

WATERS, E.J. – ALEXANDER, G. – MUHLACK, R. – POCOCK, K.F. – COLBY, C. – O'NEILL, B.K. – HOJ, P.B. – JONES, P. 2005. Preventing protein haze in bottled white wine. In Australian Journal of Grape and Wine Research, vol. 11, 2005, p. 215.

YAN, X. – QIAO, H. – ZHANG, H. – GUO, CH. – WANG, M. – WANG, Y. – WANG, X. 2017. Analysis of the grape (*Vitis vinifera* L.) thaumatin-like protein (TLP) gene family and demonstration that TLP29 contributes to disease resistance. In Scientific Reports, vol. 7, 2017, no. 4269. https://doi.org/10.1038/s41598-017-04105-w

ŽIAROVSKÁ, J. – FIALKOVÁ, V. – ZAMIEŠKOVÁ, L. – BILČÍKOVÁ, J. – ZELEŇÁKOVÁ, L. – KAČÁNIOVÁ, M. 2019. Expression pattern of thaumatin in the selected red varieties of *Vitis vinifera*, L. In Potravinárstvo, vol. 13, 2019, no. 1, pp. 547–552.

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Nitra, Slovaca Universitas Agriculturae Nitriae, 2020, pp. 35–39

ANALYSIS OF VIROME BY HIGH-THROUGHPUT SEQUENCING REVEALED MULTIPLE INFECTION AND INTRA-VIRUS DIVERSITY IN A SINGLE GRAPEVINE PLANT

Miroslav GLASA[1]*, Lukáš PREDAJŇA[1], Nina SIHELSKÁ[1], Katarína ŠOLTYS[2], Ana-Belén RUIZ-GARCÍA[3]

[1]Biomedical Research Centre of the Slovak Academy of Sciences, Institute of Virology, Bratislava, Slovak Republic
[2]Comenius University in Bratislava, Bratislava, Slovak Republic
[3]Instituto Valenciano de Investigaciones Agrarias, Center of Plant Protection and Biotechnology, Moncada, Spain

The ribosomal-depleted total RNA from white-berry grapevine (*Vitis vinifera*, SK933) plant showing severe chlorosis and downrolling of leaves was used for the high-throughput sequencing (HTS) analysis in order to unravel the potential contribution of the viral pathogens to the symptomatology observed. The combination of *de novo* assembly and mapping of ca. 1.1 millions of HTS reads enabled to identify and characterise a complex viral/viroid infection involving Grapevine leafroll-associated virus-2 (GLRaV-2), Grapevine leafroll-associated virus-3 (GLRaV-3), Grapevine rupestris stem pitting-associated virus (GRSPaV), Grapevine rupestris vein feathering virus (GRVFV), Grapevine Syrah virus-1 (GSyV-1) and Hop stunt viroid (HSVd). The determined nearly complete genomes of GLRaV-2 SK933 showed its high genetic divergence from previously characterised isolates. In case of GRSPaV, two variants representing different evolutionary lineages have been identified in the plant. The results further pinpoint the complexity of grapevine viral diseases and show that mixed virus infection of grapevine is rather a rule than an exception.

Keywords: *Vitis vinifera*, virus, diversity, next generation sequencing

Grapevine (*Vitis vinifera* L.) is one of the most ancient and widely grown crops in the world, used for the production of fresh fruits, wines, juices, and other by-products. On the other hand, grapevine has turned out to be the cultivated plant hosting the highest number of viral pathogens (Martelli, 2017), many of them being associated with economically important diseases, such as leafroll, rugose wood complex, leaf degeneration and flecks (Basso, Fajardo and Saldarelli, 2017).

The study of genetic diversity and the evolutionary mechanisms shaping the virus variability is important to understand virus epidemiology and emergence and is a prerequisite to design effective diagnostic tools and implement effective disease management measures.

Mutations, connected with positive and negative selection and recombination of genome are the main evolutionary forces driving the genetic diversity of viral populations. These processes, leading to the dynamic genetic structure of virus populations, have a significant role in the epidemiology of the grapevine viruses as they constitute the basis of their adaptation to the environment (Almeida et al., 2013; Maliogka et al., 2015).

A rapid, specific and effective diagnose integrated to the certification schemes is one of the most important tools to control grapevine viruses (Zherdev et al., 2018). However, the effectiveness of such measures can be negatively affected by a high virus genetic variability and occurrence of divergent variants escaping the detection (Glasa et al., 2015).

Knowledge on the occurrence of grapevine viruses in Slovakia and their characterisation remains insufficient. First studies aimed to characterize the grapevine viruses spread in Slovakia were based on standard genomic tools (Glasa, Predajňa and Komínek, 2011; Glasa and Predajňa, 2012; Predajňa et al., 2013; Predajňa and Glasa, 2016), possibly not detecting the whole viral complexity present in the grapevines. The recent developments of high-throughput sequencing (HTS) technologies and bioinformatics have drastically improved identification and characterisation of viral pathogens without prior knowledge of their primary structure (Maliogka et al., 2018). HTS has mostly had an impact so far through the identification and characterisation of new grapevine virus species, the study of diseases of unknown aetiology, with the identification of candidate disease-associated agents and, for some viruses, a large improvement of existing diagnostic assays (Saldarelli et al., 2017). In recent years, HTS has provided the possibility to identify and characterize several common or emerging grapevine viruses in Slovakia (Glasa et al., 2014; 2015; 2017; 2018).

The aim of the present work was the unbiased identification and characterization of complete virome present in a grapevine plant showing severe virus-like symptoms.

Contact address: Miroslav Glasa, Biomedical Research Centre of the Slovak Academy of Sciences, Institute of Virology, Dúbravská cesta 9, 845 05 Bratislava, Slovak Republic, tel.: +421 2 593 024 47, e-mail: Miroslav.Glasa@savba.sk

Material and method

An approximately 30-year old white-berry grapevine of unknown origin grown in the vineyard in Pezinok (western Slovakia, GPS coordinates: 48° 18' 09.8" N 17° 15' 40.7" E) and showing pronounced chlorosis and leafroll, was selected for the HTS analysis (further referred as the SK933 sample).

Total RNAs from fully developed leaves collected in August 2017 were extracted using the Spectrum Plant Total RNA Kit (Sigma Aldrich, St. Louis, MO, USA) and ribosomal RNA was removed using the Ribo-Zero rRNA Removal Kit (Illumina, San Diego, USA). The sample of ribosomal-depleted total RNA was used for double stranded cDNA synthesis using the SuperScript II kit (Thermo Fisher Scientific, Waltham, USA). The cDNA was then column-purified with the DNA Clean & Concentrator™-5 – DNA kit (Zymo Research, Irvine, USA) and quantified with the Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, USA). Subsequently, the sample was processed with the transposon-based chemistry library preparation kit (Nextera XT, Illumina, San Diego, USA). Low-cycle PCR and mutual indexing of the fragments was carried out. Fragments were purified with 1.8 × AMPure XP beads (BeckmanCoulter, USA) without size selection. The fragment size structure of the DNA library was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA). The equimolar pool of 4nM DNA libraries was denatured, diluted to 13 pM and sequenced (200-bp paired-end sequencing) on the Illumina MiSeq platform (Illumina, San Diego, CA, USA).

High-quality trimmed reads were used for *de novo* assembly and contigs aligned to the viral genomes database (Brister et al., 2015) or to the genome of *V. vinifera* (Velasco et al., 2007) using CLC Genomics Workbench 7.5 and Geneious v.8.1.9 softwares. Alternatively, the reads were mapped against the selected full-length sequences of viruses identified in the previous step to map the *de novo* assembled contigs (retrieved from www.ncbi.nlm.nih.gov).

Phylogenetic analyses and comparisons were performed using the MEGA v.7 (Kumar, Stecher and Tamur, 2016) and DnaSP v.6 (Rozas et al., 2017) softwares.

The nucleotide sequences reported in this paper have been deposited in the GenBank database under accession numbers listed in Table 1.

Results and discussion

The SK933 grapevine plant, exhibiting pronounced chlorosis and downrolling of leaves, was selected for the HTS analysis in order to unravel the potential contribution of the viral pathogens to the symptomatology observed.

The *de novo* assembly of about 1.1 million of high-quality HTS reads (average length of 164.9 bp) from this grapevine sample produced more than 20 000 contigs longer than 280 bp (of which 19 851 contigs mapped to the *V. vinifera* host). Several of the other generated contigs were of viral/viroid origin and led to the identification of a complex co-infection of the SK933 plant by Grapevine leafroll-associated virus-2 (GLRaV-2), Grapevine leafroll-associated virus-3 (GLRaV-3), Grapevine rupestris stem pitting-associated virus (GRSPaV), Grapevine rupestris vein feathering virus (GRVFV), Grapevine Syrah virus-1 (GSyV-1) and Hop stunt viroid (HSVd).

Combination of *de novo* assembly and subsequent mapping of NGS reads against genomes of representative isolates of above-mentioned pathogens retrieved from Genbank enabled to obtain nearly complete genomes for GLRaV-2, GLRaV3, GRSPaV and HSVd, as well as partial sequences of GRVFV and GSyV-1. Interestingly, in case of GRSPaV, two genetically different variants could be assembled from the sequence data.

Multiple alignment of the full-length nucleotide sequence of SK933 GLRaV-2 revealed only 72.1 to 85% identity of Slovak isolate with available GLRaV-2 genomes from different parts of the world. Phylogenetic analysis further confirmed the molecular divergence of SK933 GLRaV-2, clustering in a separate branch, most closely related to the Canadian and Chinese isolates (Fig. 1).

On the contrary, nearly full-length SK933 GLRaV-3 sequence showed low divergence, as compared to other GLRaV-3 isolates, and showed the close phylogenetic relationship with GLRaV-3 isolates from North and South American continent (Fig 2).

Table 1 List of viral/viroid pathogens identified in the SK933 from the HTS dataset (1 163 370 high quality reads, mean length 164,9 bp) and their characteristics

Virus acronym	Virus name	Genus	Family	NGS reads mapped to the reference	Genome coverage	Genbank accession numbers
GLRaV-2	Grapevine leafroll-associated virus-2	Ampelovirus	Closteroviridae	16,902	100%	MN548394
GLRaV-3	Grapevine leafroll-associated virus-3	Closterovirus	Closteroviridae	7,435	99.2%	MN548393
GRSPaV	Grapevine rupestris stem pitting-associated virus	Foveavirus,	Betaflexiviridae	1,335 860	93.3% 49.3%	MN548395a MN548396b
GRVFV	Grapevine rupestris vein feathering virus	Marafivirus	Tymoviridae	288	67.4%	not submitted
GSyV-1	Grapevine Syrah virus-1	Marafivirus	Tymoviridae	432	85.7%	not submitted
HSVd	Hop stunt viroid	Hostuviroid	Pospiviroidae	63	100%	MN548397

a) GRSPaV group 2a variant, b)group 3 variant

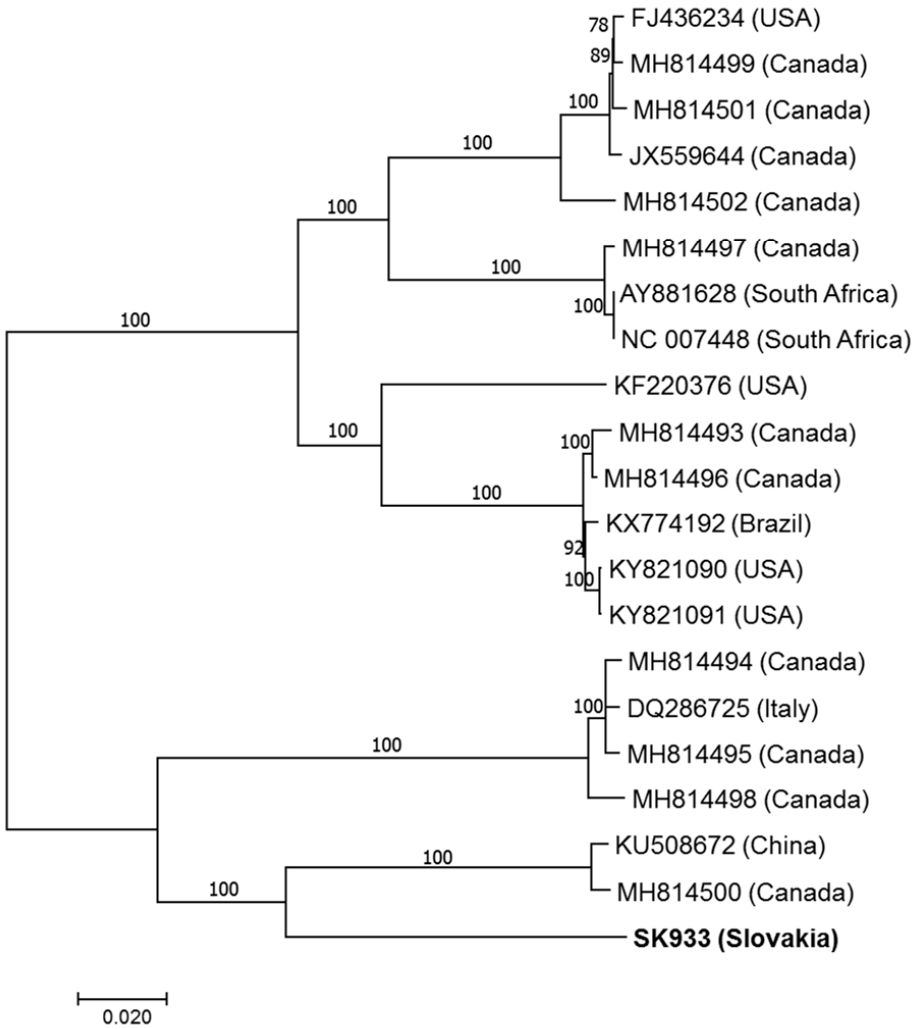

Figure 1 Phylogenetic tree generated on complete nucleotide genome sequences of GLRaV-2 isolates. Isolates are identified by their GenBank accession number and country of their origin. The Slovak isolate sequenced in the present study is highlighted in bold. The scale bar indicates a genetic distance of 0.02. Bootstrap values higher than 70% (1,000 bootstrap resamplings) are indicated

The determined full-length HSVd genome consisted of 297 nt, having the same length and 100% identity with HSVd isolates from Brazil (MF774869, MF774870, MF774873) and China (AB219944).

Two molecularly distinct variants of GRSPaV identified in the grapevine plant differed mutually by 26.8% at the nucleotide level and belonged, respectively, to the phylogenetic groups 2a and 3 (Glasa et al., 2017) and thus to different evolutionary lineages.

In case of two other viruses identified (GRVFV and GSyV-1), their genomes could not be completed because of the lower coverage of the full-length reference sequences (Table 1). However, based on the partial sequence data, both exhibited a close relationship to the respective isolates previously reported from Slovakia (Glasa et al., 2015; Glasa et al., 2019).

HTS-based characterisation of divergent GLRaV-2 variant highlights the need for a continual assessment of the grapevine virus molecular variability (also at the regional level) as a prerequisite to understand the globality of virus variability. Also, the identification of several viruses/viroids and, moreover, different variants of the same virus in a single plant, further emphasizes the complex and heterogeneous nature of grapevine viral diseases (Komínek, Glasa and Komínková, 2009; Glasa et al., 2017), indicating that a complex viral infection of grapevine is rather a rule than an exception.

The close phylogenetic clustering of geographically different isolates from different unrelated countries, or even continents (as observed e.g. for GLRaV-2, GLRaV-3 and HSVd) suggests a long term uncontrolled spread of these pathogens and their widespread dissemination, probably through the exchange and trade of infected propagation material.

The aetiology of the disease observed in the SK933 plant cannot be elucidated properly, as the coexistence of several virus/viroid agents in a single grapevine plant (possibly acting in synergy or antagonism) challenges the establishment of links between the symptoms observed and the presence of a given infectious agent.

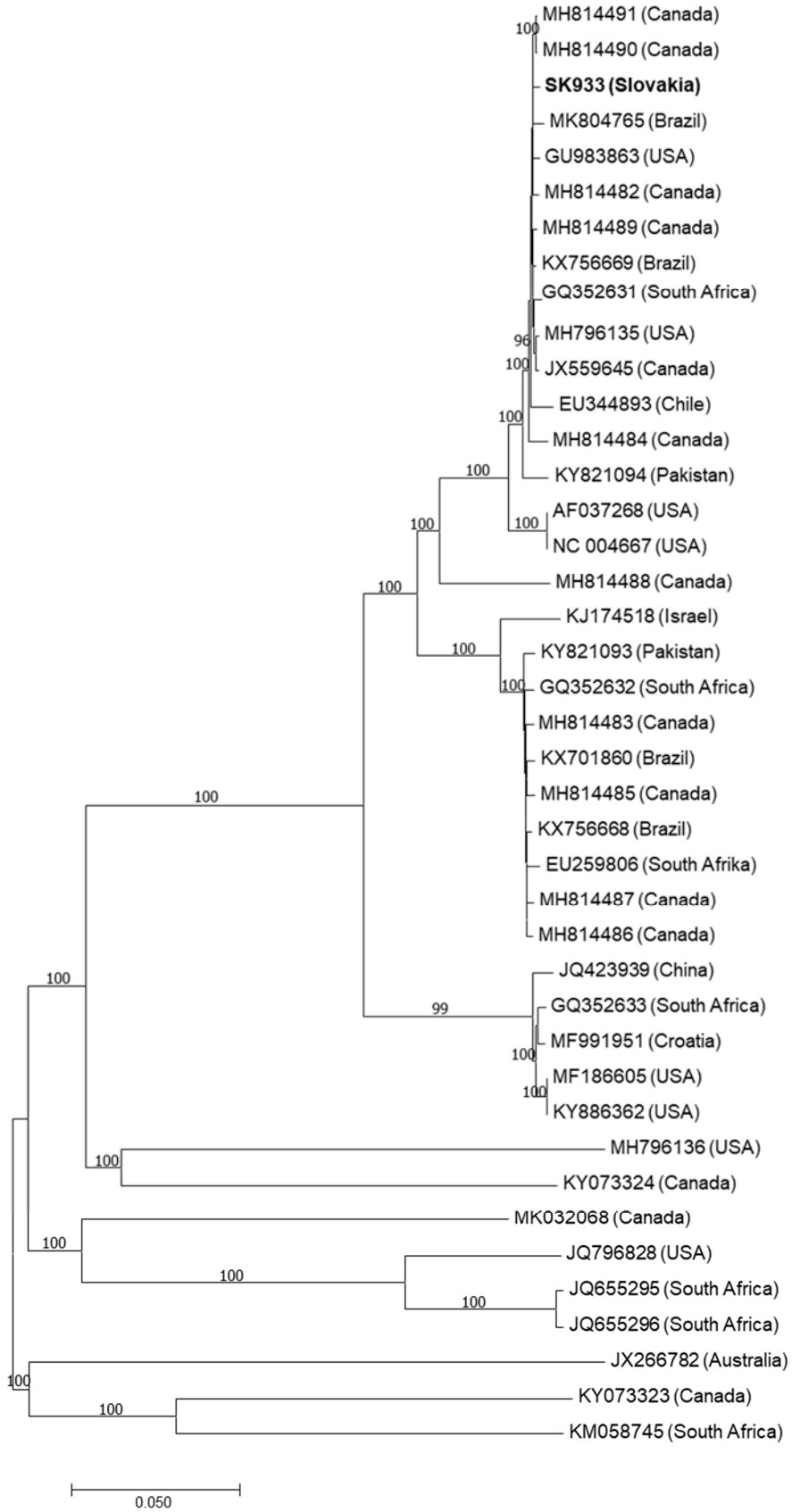

Figure 2 Neighbour-joining phylogenetic tree generated from nearly complete GLRaV-3 sequences. The corresponding sequences of previously characterized isolates are identified by their accession numbers and geographical location. Only bootstrap values ≥70% (1,000 bootstrap resamplings) are indicated. The scale bar indicates a genetic distance of 0.05

HTS technologies have been confirmed to be a powerful diagnostic tool allowing for an exhaustive description of viral species present in many grapevines (Saldarelli et al., 2017). Further information about viromes of grapevines from different production areas or agroecological contexts should help to improve the control and analyses of the different interactions involved in the ecology and pathogenicity of viral agents. This is particularly important for novel viral agents, recently identified by HTS. Moreover, in case of already known viruses, gaining access to the unbiased viral diversity might allow to validate and further improve existing detection assays by fine tuning the detection, e.g. through designing updated primers in order to improve their polyvalence and/or specificity.

Conclusion

In this work, ribosomal-depleted total RNA isolated from leaves of symptomatic grapevine plant grown in a vineyard in western Slovakia was subjected to HTS. Analysis of obtained sequence dataset revealed the presence of complex viral/viroid infection involving members of the Closteroviridae, Betaflexiviridae, Tymoviridae and Pospiviroidae families. Moreover, in case of GRSPaV, two genetically distinct variants were identified. Together, these results further highlight the complex and heterogeneous nature of grapevine virome, hampering a clear-cut establishment of links between the symptoms observed and the respective infectious agent(s) present in the plant.

Acknowledgements

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References

ALMEIDA, R.P. – DAANE, K.M. – BELL, V.A. – BLAISDELL, G.K. – COOPER, M.L. – HERRBACH, E. – PIETERSEN, G. 2013. Ecology and management of grapevine leafroll disease. In Frontiers in Microbiology, vol. 4, 2013, pp. 94, doi:10.3389/fmicb.2013.00094.

BASSO, M.S. – FAJARDO, T.V.M., – SALDARELLI, P. 2017. Grapevine virus diseases: economic impact and current advances in viral prospection and management. In Revista Brasileira de Fruticultura, vol. 39, 2017, e-411, doi: 10.1590/0100-29452017411

BRISTER, J.R. – AKO-ADJEI, D. – BAO, Y. – BLINKOVA, O. 2015. NCBI Viral Genomes Resource. In Nucleic Acids Research, vol. 43, 2015, pp. 571–577. doi: 10.1093/nar/gku1207

GLASA, M. – PREDAJŇA, L. 2012. Partial sequence analysis of a grapevine leafroll-associated virus 3 isolate from Slovakia. In Journal of Plant Pathology, vol. 94, 2012, pp. 675–679.

GLASA, M. – PREDAJŇA, L. – KOMÍNEK, P. – NAGYOVÁ, A. – CANDRESSE, T. – OLMOS, A. 2014. Molecular characterization of divergent grapevine Pinot gris virus isolates and their detection in Slovak and Czech grapevines. In Archives of Virology, vol. 159, 2014, pp. 2103–2107.

GLASA, M. – PREDAJŇA, L. – KOMÍNEK, P. 2011. Grapevine fleck virus isolates split into two distinct molecular groups. In Journal of Phytopathology, vol. 159, 2011, pp. 805–807.

GLASA, M. – PREDAJŇA, L. – SIHELSKÁ, N. – ŠOLTYS, K. – RUIZ-GARCÍA, A.B. – OLMOS, A. – WETZEL, T. – SABANADZOVIC, S. 2018. Grapevine virus T is relatively widespread in Slovakia and Czech Republic and genetically diverse. In Virus Genes, vol. 54, 2018, pp. 737–741.

GLASA, M. – PREDAJŇA, L. – ŠOLTYS, K. – SABANADZOVIC, S. – OLMOS, A. 2015. Detection and molecular characterisation of Grapevine Syrah virus-1 isolates from Central Europe. In Virus Genes, vol. 51, 2015, pp. 112–121.

GLASA, M. – PREDAJŇA, L. – ŠOLTYS, K. – SIHELSKÁ, N. – NAGYOVÁ, A. – WETZEL, T. – SABANADZOVIC, S. 2017. Analysis of Grapevine rupestris stem pitting-associated virus in Slovakia reveals differences in intra-host population diversity and naturally occurring recombination events. In The Plant Pathology Journal, vol. 33, 2017, pp. 34–42.

GLASA, M. – PREDAJŇA, L. – WETZEL, T. – ŠOLTYS, K. – SABANADZOVIC, S. 2019. First report of Grapevine rupestris vein feathering virus in grapevine in Slovakia. In Plant Disease, vol. 103, 2019, pp. 170.

KOMÍNEK, P. – GLASA, M. – KOMÍNKOVÁ, M. 2009. Analysis of multiple virus-infected grapevine plant reveals persistence but uneven virus distribution. In Acta Virologica, vol. 53, 2009, pp. 281–285.

KUMAR, S. – STECHER, G. – TAMURA, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. In Molecular Biology and Evolution, vol. 33, 2016, pp. 1870–1874.

MALIOGKA, V.I. – MARTELLI, G.P. – FUCHS, M. – KATIS, N.I. 2015. Control of viruses infecting grapevine. In Advances in Virus Research, vol. 91, 2015, pp. 175–227.

MALIOGKA, V.I. – MINAFRA, A. – SALDARELLI, P. – RUIZ-GARCÍA, A.B. – GLASA, M. – KATIS, N. – OLMOS, A. 2018. Recent advances on detection and characterization of fruit tree viruses using high-throughput sequencing technologies. In Viruses, vol. 10, pii: E436, doi: 10.3390/v10080436

MARTELLI, G.P. 2017. An overview on grapevine viruses, viroids, and the diseases they cause. In Grapevine Viruses: Molecular Biology, Diagnostics and Management (Meng, B. – Martelli, G.P. – Golino, D.A. – Fuchs, M. eds.)- Springer International Publishing, 2017, pp. 31–46. doi: 10.1007/978-3-319-57706-7_2

PREDAJŇA, L. – GAŽIOVÁ, A. – HOLOVIČOVÁ, E. – GLASA, M. 2013. Analysis of a short genomic region of Grapevine leafroll-associated virus 1 (GLRaV-1) reveals the presence of two different molecular groups of isolates in Slovakia. In Acta Virologica, vol. 57, 2013, pp. 353–356.

PREDAJŇA, L. – GLASA, M. 2016. Partial sequence analysis of geographically close Grapevine virus A isolates reveals their high regional variability and an intra-isolate heterogeneity. In Journal of Phytopathology, vol. 164, 2016, pp. 427–431.

ROZAS, J. – FERRER-MATA, A. – SÁNCHEZ-DELBARRIO, J.C. – GUIRAO-RICO, S. – LIBRADO, P. – RAMOS-ONSINS, S.E. – SÁNCHEZ-GRACIA, A. 2017. DnaSP 6: DNA Sequence Polymorphism Analysis of large data sets. In Molecular Biology and Evolution, vol. 34, 2017, pp. 3299–3302.

SALDARELLI, P. – GIAMPETRUZZI, A – MAREE, H – AL RWAHNIH, M. 2017. High-Throughput Sequencing: Advantages beyond virus identification. In Grapevine Viruses: Molecular Biology, Diagnostics and Management (Meng, B. – Martelli, G.P. – Golino, D.A. – Fuchs, M. eds.), Springer International Publishing, 2017, pp. 625–642, doi: 10.1007/978-3-319-57706-7_30

VELASCO, R. – ZHARKIKH, A. – TROGGIO, M. – CARTWRIGHT, D.A. – CESTARO, A. et al. 2007. A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. In PLoS One, vol. 2, 2007, E1326. doi: 10.1371/journal.pone.0001326

ZHERDEV, A.V. – VINOGRADOVA, S.V. – BYZOVA, N.A. – POROTIKOVA, E.V. – KAMIONSKAYA, A.M. – DZANTIEV, B.B. 2018. Methods for the diagnosis of grapevine viral infections: A review. In Agriculture, vol. 8, 2018, pp. 195; doi: 10.3390/agriculture8120195.

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THE EFFECT OF THE STORAGE ON THE CONTENT OF THE MALVIDIN-3-GLUCOSIDE IN RED WINE

Ivo SOURAL[1]*, Petr ŠNURKOVIČ[1], Eva TOMÁNKOVÁ[1], Astrid FORNECK[2]

[1]Mendel University in Brno, Czech Republic
[2]University of Natural Resources and Life Sciences, Austria

Anthocyanins are plant dyes responsible for the colour of red wine. Of these, malvidin-3-glucoside is the most significant member and its content was monitored in wines that were first left to age in oak barrels for 12 months, subsequently bottled and sealed with plastic/cork stoppers. The malvidin-3-glucoside content was also studied in the same wines that were bottled right away without aging in barrels. Analyses were conducted within the time spans of 3 to 30 months. The highest malvidin-3-glucoside concentrations were found in month 3 of the aging process, and they significantly decreased in month 6 and month 15 of storing. Between month 15 and month 30 of storing, the content of malvidin-3-glucoside basically remained unchanged. The results of the measurements show that to preserve higher malvidin-3-glucoside levels for longer periods of time, it is better to store wine in barrels rather than in bottles and when a bottle is used instead of a barrel, a plastic stopper is better than a cork stopper.

Keywords: wine, malvidin-3-glucoside, storage, barrel aging, plastic/cork stopper

Anthocyanins and derivatives thereof are essential pigments responsible for the colouring of red wine (He et al., 2012). Anthocyanins are dyes of a plant origin that accumulate in the hypodermal cell layer of the skin of berries of the *Vitis* genus as they ripen. Rather high anthocyanin levels in grapes correlate with a quantity of sugars (Soural et al., 2015). The dyes are released from the skins of grapes during the process of vinification. Structurally, anthocyanins are heteroglycosides that consist of the sugar component and aglycone (anthocyanidin). Anthocyanins are rather unstable and are subject to a degradation process, which is influenced by a number of factors such as temperature, light, level of oxygen etc. (Mezey et al., 2016). Monomeric anthocyanins, with their colour being pH-dependent (Tománková et al., 2016), are responsible for the colour of young red wines, and they become highly unstable when their concentrations rapidly fall and they become involved in the production of much more stable oligomeric and polymeric structures as red wines age (Fulcrand et al., 2006). These new colourful compounds are entirely responsible for the colouring of rather old red wines; they have also become less sensitive to changed pH and more resistant to discolouring by sulphur dioxide by that time (Somers, 1971). Malvidin-3-glucoside is the substance most frequently occurring in young red wines; malvidin is found as a monoglucoside and is present already in blue grapes (Čopíková et al., 2005). Balík, Kumšta and Rop (2013) even report that out of more than 20 types of anthocyanins, malvidin-3-glucoside constitutes over 60% of all anthocyanins in Blaufränkisch as well as other grape varieties.

Material and method

Wine

Two wines (Blaufränkisch & Cuvee from the wine region Moravia, GPS of vineyard: 48° 51' 54.0" N, 16° 53' 24.0" E) aged in oak barrels, 50 litres of each, for 12 months, subsequently were decanted using 0.7 l bottles; plastic (P) and cork (C) stoppers were used, each having a length of 45 mm and a diameter of 25 mm. In parallel, the same wines were bottled straight away without being retained in barrels, using again cork (C) or plastic (P) stoppers.

The wines were stored in a wine cellar in the municipality Velké Bílovice in the Czech Republic in the dark and at a temperature of 12 °C and analysed for the level of malvidin-3-glucoside (MvGl), in the course of 3 to 30 months. On a day before taking the measurements, the wines were placed in a refrigerator at 5 °C. The analysis was carried out in month 3, 6, 15, 21 and 30 of aging on Faculty of Horticulture in Lednice (Mendel University in Brno, Czech Republic).

Solid phase extraction (SPE)

Before applying a sample of wine, SPE columns (6 ml.500 mg^{-1} of polystyrene-divinylbenzene) were flushed with 6 ml of acidified methanol (0.01% HCl in vol. % in pure methanol at HPLC grade >99.9%) and, subsequently, with acidified water (again, 0.01% HCl in vol. %). The sample was diluted to 1 : 1 with acidified water when 6 ml was applied to the SPE column (any non-polar MvGl was absorbed). Then there was an action of washing using 6 ml of acidified water

Contact address: Ivo Soural, Mendel University in Brno, Faculty of Horticulture, Department of Post-Harvest Technology of Horticultural Products, Valtická 337, Lednice 69144, Czech Republic, e-mail: ivo.soural@mendelu.cz

to remove the polar substances. The elution of MvGl was made using 6 ml of acidified methanol.

Concentrating the samples

The eluent was made more concentrated by evaporation of the solvent (by increasing the temperature to +35 °C under inert atmosphere using a flow of gaseous nitrogen). The resulting dry matter was subsequently dissolved with 1 ml of the mobile phase A (see the Liquid chromatography chapter) and filtered using a nylon syringe filter, pore size of 0.22 μm (Membrane Solution); the solution was diluted, as necessary, with the mobile phase A for measuring liquid chromatography (LC).

Liquid chromatography

Malvidin-3-glucoside was analysed using LC (Thermo Electron, Finnigan ChromQuest) on a column Synergy (Phenomenex, Torrance, CA, USA) with parameters: 5 μm, 250 mm×4.6 mm, the temperature of 35 °C, the flow rate of 0.5 ml.min^{-1}. The mobile phase A was 5% acetonitrile +5% formic acid (in vol. %) in water; the mobile phase B was 55% acetonitrile + 5% formic acid (in vol. %) in water. The gradient was increased from 6 % mobile phase B to 20% of B during 20 min; from 20% of B to 40% of B during 15 min; from 40% of B to 60% of B during 5 min; from 60% of B to 90% of B during 5. The injection volume was 5 μl. Diode array detector (Thermo Electron, Finnigan UV6000LP) with the detection of wavelengths at 210, 280 and 520 nm was used for analyzing. Wavelength 520 nm was used for the quantitative evaluation. For the calibration, the analytical standard malvidin-3-glucoside (from PhytoLab at HPLC grade ≥95.0%) was used (retention time 26.8 min.).

Results and discussion

As the wine aged, malvidin-3-glucoside (MvGl) levels were on the decrease. The highest values were recorded in month 3 of aging; during the subsequent months (6, 15, 24 and 30), lower values were recorded each time. The decline in MvGl was rendered by power or exponential characteristics (Fig. 1, Fig. 2a and details in Fig. 2b) rather than by the linear one (for example, the regression coefficient seen for the MvGl level in Blaufränkish wines stored in bottles with plastic stoppers was R = 0.9243 for the power characteristic, R = 0.8340 for the exponential characteristic, and only R = 0.7970 for the linear characteristic). A rapid decrease in monomeric anthocyanins was observed by Zafrilla et al. (2003) as well.

For Cuvee, the average MvGl levels in month 3 of storing were around 35 mg.l^{-1} (34.3 for cork; 38.0 for plastic and 36.3 for barrels); in month 6, they were around 16 mg.l^{-1} for wines stored in bottles (15.8 mg.l^{-1} under cork stoppers and 16.0 mg.l^{-1} under plastic stoppers), while in barrels, the content was significantly higher (26.8 mg.l^{-1}, which is approximately 2/3 higher than in bottles; see Fig. 1). A similar situation occurred in the subsequent months when in month 15, 24 and 30 the Cuvee's MvGl concentrations were around 3 mg.l^{-1} when stored in bottles with cork/plastic stoppers; however, after 12 months of aging in barrels and subsequent bottling (using cork/plastic stoppers), Cuvee had the MvGl concentration of around 6 mg.l^{-1}, i.e., roughly twice as much (Fig. 1).

Similar trends in the course of the MvGl concentration were found in Blaufränkisch (Fig. 2a). However, in month 3 of storing, there was approximately 70 mg.l^{-1} of MvGl in the barrel, while in bottles (both types of stoppers), there was only around 50 mg.l^{-1} (48.9 for cork; 55.6 for plastic), which means that generally, the level in barrels was 35% higher than in bottles (45% under cork stoppers, about 28% under plastic stoppers). Within month 6, the difference was as much as nearly 40%, while in barrels there was 28.0 mg.l^{-1} of MvGl and in bottles (both types of stoppers) there was an average of 20.2 mg.l^{-1}, when, again, in wines under plastic stoppers there was a little higher concentration. The MvGl concentration in month 15 was in fact identical for all types of storage; it was around 3 mg.l^{-1}. In the subsequent months of storage, significantly higher levels were measured in the samples with 12 months of aging in barrels than in those stored only in bottles during the storage period. For the wine that aged in barrels for 12 months and was stored under cork and plastic stoppers for another period of 12 months, the MvGl levels were 12.8 and 11.4 mg.l^{-1}, respectively, while the wine stored under stoppers for 24 months contained only 4.6 mg.l^{-1} (cork) and 3.0 mg.l^{-1} (plastic). This hence, practically, constituted 4-fold differences in terms of the MvGl content. Within month 30 of storing, this ratio even increased to about an 8-fold difference between the wine stored only in bottles and the one aging in barrels for 12 months and stored in bottles for another period of 18 months.

For nine out of ten cases of Cuvee, it was characteristic that they reached lower values when stored only in bottles than when they were left aging in barrels for 12 months. The same applied to Blaufränkisch as nine out of ten cases were

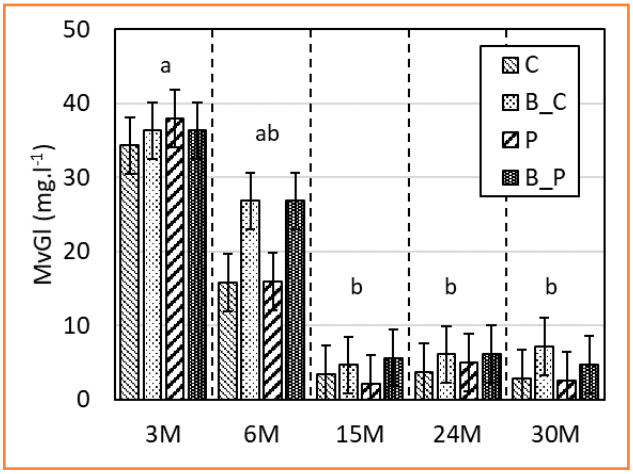

Figure 1 Distribution of malvidin-3-glucoside (MvGl) in Cuvee wine stored in bottles with cork (C) or plastic (P) stoppers during the aging period of 30 months (30M means 30 months) and the one stored in oak barrels (B) for the initial 12 months and, subsequently, in bottles with cork/plastic (C/P) stoppers; different letters (a, b) in the time of storage indicate a significant difference in contents of MvGl by Tukey test (P = 0.05); for each variant, there were 3 or 4 repetitions made

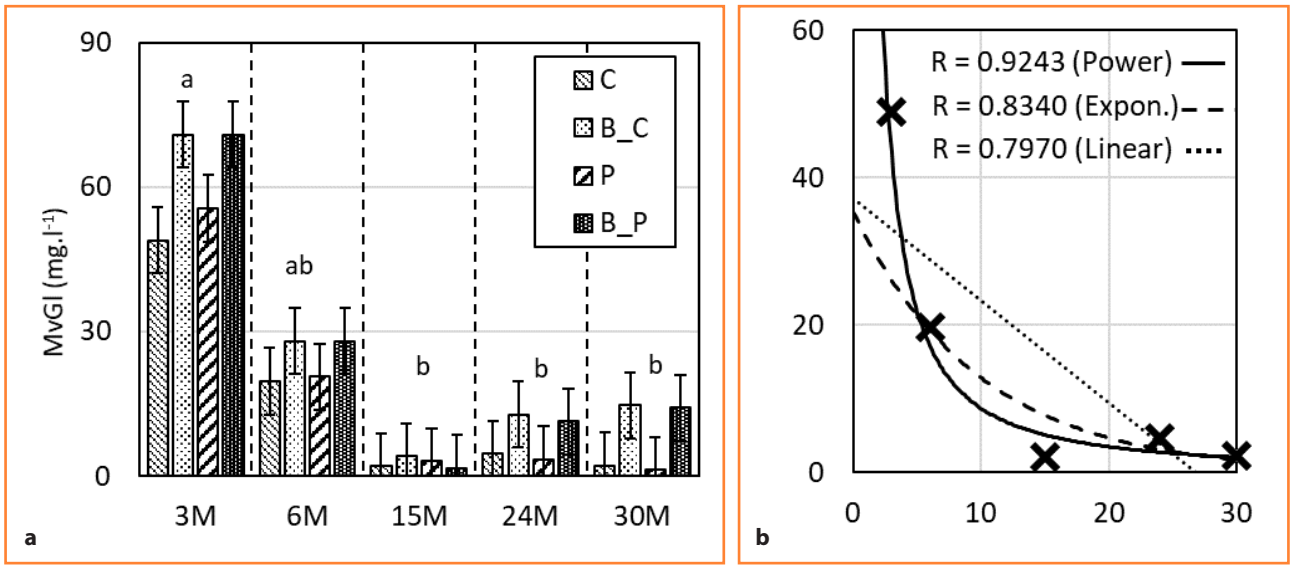

Figure 2 **a** – Distribution of malvidin-3-glucoside (MvGl) in Blaufränkisch wine stored in bottles with cork (C) or plastic (P) stoppers during the aging period of 30 months (30M means 30 months) and the one stored in oak barrels (B) for the initial 12 months and, subsequently, in bottles with cork/plastic (C/P) stoppers; different letters (a, b) in the time of storage indicate a significant difference in contents of MvGl by Tukey test ($P = 0.05$); for each variant, there were 3 or 4 repetitions made
b – In detail, (P) with 3 test curves to decrease MvGl, regression coefficients (R) were obtained by the dependence of level of MvGl on time of aging

found to have higher MvGl levels when wine was left aging in barrels.

In both Cuvee and Blaufränkisch stored under plastic stoppers, in six out of ten cases, the MvGl levels were higher than in those, found in wines under cork stoppers. In the case of Cuvee aging in barrels for 12 months, two out of three cases were observed to have higher MvGl levels compared with Cuvee wine stored under plastic stoppers, whereas the opposite was true for Blaufränkisch when three out of three cases were found to have lower MvGl levels when stored under plastic stoppers compared with those using cork; the differences, however, were significantly below 2.5 mg.l^{-1}.

Zafrilla et al. (2003) monitored MvGl levels in wine for the Monastrell variety stored in glass bottles in the dark when the concentration was 49.9 mg.l^{-1} for the conventional wine and 75.9 mg.l^{-1} for the ecological wine in month 3. The levels became reduced when they reached 39.2 mg.l^{-1} (a 21% decrease) and 47.1 mg.l^{-1} (a 38% decrease) in month 6. As a part of our measurements made between month 3 and 6 of storing, the reduction of 26% was recorded in Cuvee when stored in barrels (i.e., in the dark); for storing in bottles, however, a decrease of more than 50% was recorded for the same wine stored in bottles. Concerning Blaufränkisch wine, the reduction ranged from 59% to 63% for all of the storing options. The declines between month 3 and 6 of storing are therefore in tens of %.

The reduction of all anthocyanins (i.e., not only that of MvGl) during the storage was measured previously by Mazza et al. (1999) when the total quantity of anthocyanins expressed as MvGl declined by 25% (from 469 mg.l^{-1} to 352 mg.l^{-1}) after 2 months for Cabernet Franc, by 22% (from 455 mg.l^{-1} to 355 mg.l^{-1}) after 6 months for Merlot, and by 25% (from 219 mg.l^{-1} to 166 mg.l^{-1}) after 5 months for Pinot Noir.

A similar percentual decline (by 20%) was recorded by Gómez-Plaza et al. (2002) when they determined, for the Monastrell variety stored in bottles, the malvidin level to be 87.9 mg.l^{-1} in month 3 and 70.1 mg.l^{-1} in month 6. In addition, they measured the content of 41.1 mg.l^{-1} even in month 12. However, this was a decrease of 53% compared with month 3. For the samples measured by us in month 15, the declines ranged between 84% and 95% for Cuvee and between 94% and 98% for Blaufränkisch. During one-year period of the storage, the declines are already several tens of percent.

Conclusion

The present study compared four methods of a wine-storing: in bottles with plastic (P) or cork (C) stoppers, aging in oak barrels for initial 12 months and subsequent bottling using stoppers of plastic (B_P) or cork (B_C), and the effect of these on the malvidin-3-glucoside (MvGl) content. The effect was studied during the storage period from 3 to 30 months in the case of the Blaufränkisch and Cuvee varieties. The decline of MvGl, concerning time, was rendered by the power ($R = 0.9243$) or exponential ($R = 0.8340$) characteristics rather than by the linear one ($R = 0.7970$) as shown by the values for Blaufränkish (P). This shows a rapid decline in MvGl in the early days of the storage when after month 15, the MvGl quantities practically remained unchanged. In 18 out of 20 cases, the MvGl levels were higher in wines stored in barrels for the initial 12 months compared with those stored in bottles. For wines aging in bottles only, the MvGl levels were higher in six out of ten cases when wines were stored under plastic stoppers compared with those stored under cork stoppers. Between month 3 and month 6 of storing, the reduction of 26% was recorded for Cuvee stored in barrels; in the case of storing the same variety in bottles, however, even more than 50% reduction was observed. For Blaufränkisch,

the reduction ranged between 59% and 63% for all storing options. The declines measured between month 3 and month 6 therefore reached tens of percent. When stored in one-year period, instances of the MvGl level reduction were extremely significant; for example, they ranged, from month 3 to month 15, between 84% and 95% and between 94% and 98% for Cuvee and Blaufränkisch, respectively. Hence, the results of the measurements show that to preserve the higher levels of MvGl for a longer period, it is better to store wine in barrels than in bottles and when one does use bottles instead of barrels, plastic stoppers are better for use than cork stoppers.

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References

BALÍK, J. – KUMŠTA, M. – ROP, O. 2013. Comparison of anthocyanins present in grapes of *Vitis vinifera* L. varieties and interspecific hybrids grown in the Czech Republic. In Chemical Papers, vol. 67, 2013, no. 10, pp. 1285–1292.

ČOPÍKOVÁ, J. – UHER, M. – LAPČÍK, O. – MORAVCOVÁ, J. – DRAŠAR, P. 2005. Přírodní barevné látky. In Chemické listy, vol. 99, 2005, pp. 802–816.

GÓMEZ-PLAZA, E. – GIL-MUÑOZ, R. – LÓPEZ-ROCA, J. M. – MARTÍNEZ-CUTILLAS, A. – FERNÁNDEZ-FERNÁNDEZ, J. I. 2002. Maintenance of Colour Composition of a Red Wine During Storage. Influence of Prefermentative Practices, Maceration Time and Storage. In Lebensmittel-Wissenschaft & Technologie, vol. 35, 2002, pp. 46–53.

FULCRAND, H. – DUENAS, M. – SALAS, E. – CHEYNIER, V. 2006. Phenolic reactions during winemaking and aging. In American Journal of enology and viticulture, vol. 57, 2006, no. 3, pp. 289–297.

HE, F. – LIANQ, N. N. – MU, L. – PAN, Q. H. – WANG, J. – REEVES, M. J. – DUAN, C. Q. 2012. Anthocyanins and their variation in red wines. II. Anthocyanin derived pigments and their color evolution. In Molecules, vol. 17, 2012, no. 2, pp. 483–519.

MAZZA, G. – FUKUMOTO, L. – DELAQUIS, P. – GIRARD, B. – EWERT, B. 1999. Anthocyanins, Phenolics, and Color of Cabernet Franc, Merlot, and Pinot Noir Wines from British Columbia. In Journal of Agricultural and Food Chemistry, vol. 47, 1999, pp. 4009–4017.

MEZEY, J. – CZAKO, P. – MEZEYOVÁ, I. – BAJČAN, D. – KOBOLKA, R. 2016. Changes of Selected Antioxidant Parameters of Red Wines during Maturation. In Czech Journal of Food Science, vol. 34, 2016, no. 4, pp. 356–361.

SOMERS, T.C. 1971. The polymeric nature of wine pigments. In Phytochemistry, vol. 10, 1971, pp. 2175–2186.

SOURAL, I. – BALÍK, J. – WENDELIN, S. – EDER, R. 2015. Analyses of Compounds in Skins, Pulps and Whole Berries of Thirty-Three Austrian Table Grape Cultivars. In Acta Horticulturae, vol. 1079, 2015, pp. 527–534.

TOMÁNKOVÁ, E. – BALÍK, J. – SOURAL, I. – BEDNÁŘ, P. – PAPOUŠKOVÁ, B. 2016. Colour and antioxidant properties of malvidin-3-glucoside and Vitisin A. In Acta Alimentaria, vol. 45, 2016, no. 1, pp. 85–92.

ZAFRILLA, P. – MORILLAS, J. – MULERO, J. – CAYUELA, J. M. – MARTÍNEZ-CACHÁ, A. – PARDO, F. – LÓPEZ NICOLÁS, J. M. 2003. Changes during Storage in Conventional and Ecological Wine: Phenolic Content and Antioxidant Activity. In Journal of Agricultural and Food Chemistry, vol. 51, 2003, no. 16, pp. 4694–4700.

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A REVIEW: A FRAMEWORK FOR YIELD IMPROVEMENT IN KHARIF/RAINY SEASON POTATO IN THE LOW LAND TROPICS

Shankar RAJA*, Govindakrishnan PM, Chakrabarti SK

ICAR-Central Potato Research Institute-Shimla, India

Potato is a temperate crop greately adapted to tropical climate as evidenced by the greater share of production by the tropical countries in recent years. It is grown mainly during rainy seasons coupled with long summers in majority of potato growing countries. However, the sub-tropics country like India, *kharif* (or) rainy season potatoes is still an underexploited segment which is mainly restricted to hills and plateaus, but not plains. Unlike short day crops grown during winter, the yield of *kharif* potato is far lower. However, the simulation model estimated the attainable yield could be enhanced substantially by extending canopy cover (100%) duration from 10 to and 40 days over the existing window of favourable growing period at various *kharif* growing areas. Accordingly, the yield could be enhanced from 25.4 to 34.7 t.ha^{-1} in plateaus (Dharwad), and from 31.9 to 42.0 t.ha^{-1} in Northern HH (Shimla). The South and Northern HH locations (Ooty and Shimla respectively) registered a higher attainable yield (45.1 and 42.0 t.ha^{-1}, respectively) which strongly indicates the necessity of trait specific improvement program for developing better phenotype combination having high water, radiation and light use efficiency for enhanced yield potential.

Keywords: environment, future research, genotype, *kharif* potato, physiological breeding

Potato (*Solanum tuberosum* L.) is considered to be the 3rd most important food crop in the world. It is grown in >125 countries comprising 52% areas located in temperate region in Europe, 34% in Asia and 14% in Africa. The world potato production is estimated at 364.8 million t in 2012 (FAO, 2014), where the production share of the developing world exceeded the developed (Scott and Suarez, 2011. Potato is grown in majority of leading potato growing countries during rainy seasons under long summer which results in higher productivity (Table 1). India is the 2nd largest producer of potato in the world (Scott and Suarez, 2011) by producing 45.34 million t from 1.99 million ha (22.8 t.ha^{-1}) during 2012–2013 (NHB, 2013). *Kharif* crop is grown during rainy seasons in hills and plateaus recorded with very low productivity. In India, plains shared about 85% crop area and rest (15%) is contributed by hills and plateaus. Considering the country future demand of potato (125 million t), the area under potato is required to increase to 3.62 million ha to meet this demand (CPRI, 2011). Exploitation of not only hills and plateau areas, but also extending areas under potato cultivation to plains during rainy seasons is indispensable. In order to analyse climatic normals of different *kharif* growing regions, the climatic data of Indian Meteorological Department for 15 years was collected and used. LINTUL-POTATO-DSS model was used to estimate the predicted yield of potato for *kharif* growing area (Haverkort et al., 2015). Hence, this review explores the prospects of *kharif* potatoes and future strategies for its exploitation in the low land tropics like the Indian subcontinent.

Kharif potato scenario in India

The word *Kharif* denotes "Mousam" originated from Arabic; hence, it is most popular in Indian sub continent and Arabian countries. The *kharif* potato refers to the potato crop cultivated during rainy seasons (June to September), which is most prevealent in plateaus areas (Table 2). Although planting of potato in hills has taken place earlier than onset of rains, the crop is fully dependent on forthcoming rainy seasons for completing its life cycle. Unlike short day potato grown in plains during winter season (irrigated), *kharif* potato is completely grown as a rainfed crop under long day conditions, often encountering both moisture and nutritional stresses. The vigourous vegetative, late stolon formation, and stolon growth longer under long day conditions reflects the poor yield potential as compared to the short day crop (40–50 t.ha^{-1}).

Topographic scenario of *kharif* potato growing areas

The altitude based classification of potato growing regions revealed that the crop season and crop duration of potato is location-specific (Pushkarnath, 1976). As *kharif* potato is grown both in hills and plateaus, the Indian hills are divided into North Indian, South Indian and North Eastern hills, which are further subdivided into Very high, High, Mid and Low hills based on the altitude (Table 3). The Northern Hills (NH) altitude ranged between 800 and 3,000 m a. s. l. Due to this, the sowing time of potato ranges from early February to end of April and the harvest time lasts from early July to end of October. In Very high and high hills, the crop is completely grown under rain watering and the available crop period is longer. In Mid hills, even the *kharif* crop is

Contact address: Shankar Raja, ICAR-Indian Institute of Horticutural Research, Bengaluru-560089, e-mail: Raja.Shankar@icar.gov.in

planted in February to March, there is enough soil moisture for germination and growth, but (May-June) irrigation is always essential to save the crop during dry months. Hence, the premature crop harvesting is practiced due to moisture stress which causes poor storability of tubers causing troublies with economical values. In low hills, no *kharif* crop is grown. The Southern hills (SH) altitude range rises steeply even >2,500 m a.s.l. at some point from the plains, they are located near 10° North of equatorial line and well fixed in the tropic of cancer. Potato is grown thrice a year (*Kharif* or summer/ autumn/spring) at >2,000 m a.s.l. elevations. As the rainy season starts during June, the crop is raised

Table 1 Top 20 Potato producing countries in the world and their seasonal trends

Sl. No.	Countries	Production (tonnes)	Growing period		
			March–September	December–May	October–April
1	China	85,920,000	mostly	meager	meager
2	India	45,000,000	meager	meager	mostly
3	Russian Federation	29,532,530	mostly	meager	meager
4	Ukraine	23,250,200	mostly	meager	meager
5	USA	19,165,865	mostly	meager	meager
6	Germany	10,665,600	mostly	meager	meager
7	Poland	9,091,900	mostly	meager	meager
8	Bangladesh	8,205,470	meager	meager	mostly
9	Netherlands	6,765,618	mostly	meager	meager
10	France	6,340,807	mostly	meager	meager
11	Iran	5,400,000	mostly	meager	meager
12	Turkey	4,822,000	mostly	meager	meager
13	Canada	4,590,296	mostly	meager	meager
14	United Kingdom	4,553,000	mostly	meager	meager
15	Egypt	4,500,000	meager	meager	mostly
16	Peru	4,473,503	meager	meager	mostly
17	Algeria	4,219,476	mostly	meager	meager
18	Pakistan	4,104,400	meager	meager	mostly
19	Brazil	3,731,798	meager	meager	mostly
20	Malawi	3,255,780	meager	meager	mostly

Year: 2012. Source: FAOSTAT, 2014

Table 2 Climatic characters of *kharif* potato compared with subtropical and temperate

Parameters	Subtropics	Temperate	*Kharif*
Growing season	winter	summer	rainy
Temp. at planting	25–30 °C	15–25 °C	25–30 °C
Temp. at harvesting	10–20 °C	15–25 °C	20–25 °C
Crop duration (days)	90–100	150–180	120–150
Mid day water stress	prominent	absent	present
Night temp.	4–15 °C	15 °C	18–20 °C
Frosting	common	absent	absent
Resulting yield	low	high	very low
Dry matter content	less	high	high
Growing altitude	plain	high hill	mid/high hills
Sun shine	moderate	high	moderate
Growing period	October–February	April–September	May–October

Table 3 Topographical factors of hills and plateaus regions of potato growing zones in India

Hill categories	Representative location	Altitude	Latitude	Longitude	Growing season
Northern Hills					
Very High Hills	Kufri	–	–	–	–
High Hills	Shimla	2,202	31.06	77.10	15th April – 30th Oct
Mid Hills	Almora	1,585	32.08	76.32	15th April – 15th Oct
Low Hills	Srinagar	564	32.18	75.55	30th June – 30th Sep
Southern Hills					
Very High Hills	–	–	–	–	–
High Hills	Ooty	2,249	11.24	76.44	15th April – 15th Oct
Mid Hills	Gudalore	1,052	11.30	76.30	10th April – 15th Oct
Low Hills	Mysore	767	11.18	76.57	5th April – 10th Sep
North Eastern Hills					
Very High Hills	–	–	–	–	–
High Hills	Shillong	1,598	25.34	91.53	7th March – 30th July
Mid Hills	Aijel	1,097	23.44	92.43	30th Feb – 25th July
Low Hills	Halflong	682	25.10	93.12	10th Feb – 10th July
Plateaus					
High altitude	Chigmangalur	1,018	13.20	75.46	1st May – 30th Sep
	Hassan	967	12.58	75.59	30th May – 30th Sep
	Dharwad	727	15.27	75	10th June – 5th Oct
Low altitude	Pune	559	18.32	73.51	10th June – 15th Sep

during 2nd week of April to the last week of September. However, rainfall is a basic requirement to complete the life cycle of potato in this region. The North-Eastern hills (NEH) are characterised with flat plains, undulating hillocks, hills, high plateau and mountains. In hilly areas, the potato is raised as a summer (March-July), autumn (August-December) crop where yields are generally higher in a former crop season. The high snow melts followed by rainfall take care of crop at high and very high hills. The mid and low hills completely depend on moisture through rainfall during June to September. In contrast to the very high and high hills, the growing season is warmer in mid and low hills, and therefore the crop duration is comparatively shorter.

Plateau region covers a vast area of central and peninsular India. The altitude range is from 300 to 1,200 m a.s.l., which are further classified into low altitude plateau (LAP) (300 to 600 m a.s.l.) and high altitude plateau (HAP) (600 to 1,200 m a.s.l.). In the HAP, two growing seasons (*Kharif* and rabi season) are feasible, while in the LAP only the winter season crop is possible. Crop season starts with the onset of pre-monsoon showers (early June) and extends up to the cessation of rains (end of September to October); hence, the growing season is fairly long. Under plateau region, the availability of potato growing days increases as the elevation increased. The low saltitude location Pune had very low days as compared to high altitude locations.

Climatic and phenological siutations

Temperature

Temperature plays a key role in determining the sowing time and consequently the duration of different phenol-phases which greatly affects the crop productivity. The climatic normals recorded for the crop season at hills and plateaus revealed that the maximum seasonal mean temperature had negative relation with altitude irrespective of hills (NH, SH and NEH). The mean seasonal minimum temperature found the least at NH (13.04 °C) as compared to SH (15.43 °C) and NEH (15.51 °C) clearly indicated that SH, NEH are comparatively warmer than NH. Similarly, the seasonal mean maximum temperature was found higher at NH (25.95 °C) as compared to SH (22.88 °C) and NEH (23.84 °C). In plateau regions, the mean minimum temperature observed was 19.45 °C, while the maximum mean temperature observed was 27.88 °C clearly showing a wider difference between the level of minimum and maximum temperatures between hills and plateau regions (Fig. 1).

P days and Growing degree days (GDD)

Plant development requires a specific amount of heat to develop from one point in their life cycle to another (from seeding to harvesting stage). The GDD calculated for different *kharif* grown locations for predicting phonological events more accurately (Mc Master and Wilhelm 1997) to determine harvest dates and yield revealed that the mean *p* days across altitudes were found lower for NH

(983.2 Cd) than SH (1,281.9 Cd) and NEH (1,281.2 Cd). Similarly, the *p* days calculated for plateau regions across the altitude were found lower than hills (916.2 Cd), which strongly showed a negative relation of *p* days with altitude. Growing degree (GDD) days and photo thermal units (PTU) are good estimators of growth stages. Accumulation of growing degree days and photo thermal units for each developmental stage is relatively constant and independent on the sowing date; crop variety may modify it considerably (Phadnawis and Saini 1992). The mean GDD recorded for NH was found lower (2,069.2 Cd) as compared to SH (2,313.6 Cd) and NEH (2,324.2 Cd). Similarly, GDD recorded for the plateau region was greater (2,352.8 Cd) than that of NEH and SH (Fig. 2).

Solar radiation and night temperature index

Plants' growth rate is proportional to the amount of solar radiation received for photosynthesis, assuming that other environmental parameters are not limiting. Of the 100% total energy received by the leaf, only 5% is converted into carbohydrates for biomass production. Among the representative locations, the NH observed with mean solar radiation ranged from 7.23 to 12.89 with mean value of 10.18. The SH had ranged from 12.46 to 13.24 having mean value of 12.79. The NEH recorded mean solar radiation ranging from 7.31 to 11.58 with the mean of 9.99. This data clearly indicates that SH received greater solar radiation as compared to the rest. Similarly in plateau region, the mean solar radiation ranged from 8.17 to 18.48 with mean value of 11.15. Similarly, the night temperature index observed ranged from 15.45 to 18.6 with mean value of 10.07 for NH, ranging from 12.46 to 21.22 with the mean of 17.28, and ranged from 15.87 to 19.17 with mean value of 17.56. However, it ranged from 18.56 to 24.37 with a mean of 21.53 for pleateu regions clearly indicating that SH, followed by plateau region, receives comparatively higher solar radiation and night temperature as compared to NH and NEH. These factors strongly support the fact that the poor yield is attributed to high solar radiation as evidenced that every 1.8 °C rise in temperature could cause 3–10% yield reduction in wheat (You et al., 2009). This justifies that the enhanced radiation use efficiency for elevated temperature is imperative for plateau regions (Bradshaw, 2009).

Crop growth pattern

Varietal duration

Crop duration is determined by the heat units accumulated in the growing regions and the genotype ability to harvest

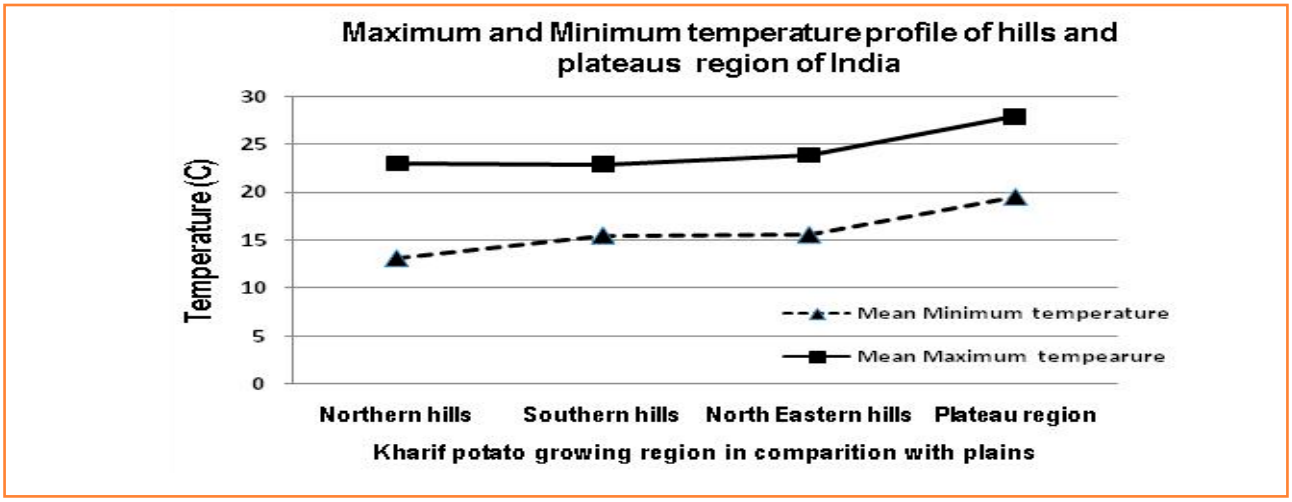

Figure 1 The mean minimum and maximum temperature profile of different *kharif* potato growing regions of India

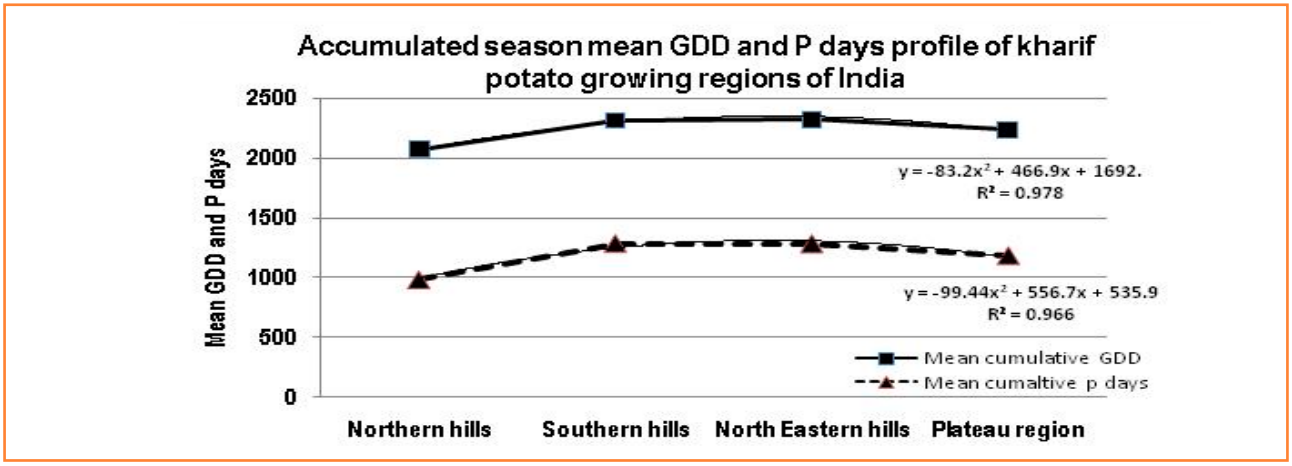

Figure 2 The growing degree days and *p* days accumulation trend of *kharif* potato growing regions of India

heat units for its growth. Although optimum temperature for potato is considered between 5 °C and 25 °C, the growth period can be widened about 10–15 days on either side of the exiting window through interventions of improved varieties developed for abiotic stress factors. Considering the suitable growing temperature for tuberisation, aerial growth and development, the volume of growing degree days was calculated for *kharif* potato locations of hills and plateau regions. The results revealed that the NH had low mean GDD (2,069.3 Cd) as compared to SH (2,313.6 Cd), NEH (2,324.2 Cd) and plateau regions (2,235.7 Cd), which is strongly supported by Hassan et al. (2007) that higher elevation areas of Canada had lower GDD at 400–800 m a.s.l. range. On the contrary, the higher GDD (2,656.8 Cd) recorded at plains (Modipuram) as compared to hills might be due to land use patterns of forest lands exhibiting relatively cooler GDD associated with higher evapotranspiration rates in forested areas (Hassan et al., 2007). Few exceptions showing that mid location of SH and NEH had greater GDD as compared to high hills of all the three groups can be justified by higher volume of non cloudy days (Hassan et al., 2007). Hence, the high hills required more periods to accumulate the required GDD to complete the life cycle of potato as compared to mid and low hills and plateaus. The lower hills and plateau regions had higher GDD values despite the shorter growing days due to higher maximum temperature during the crop season.

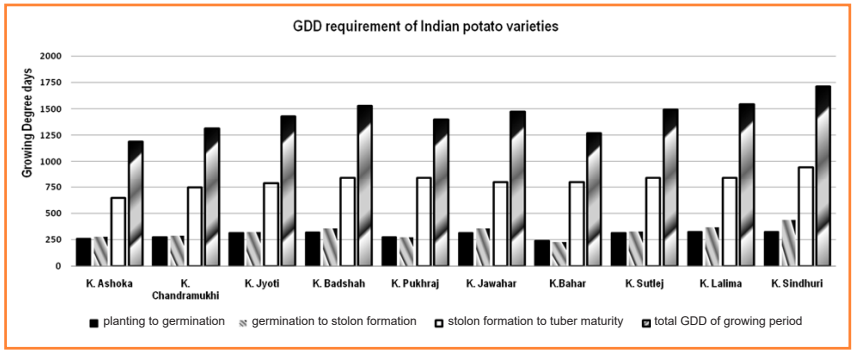

Figure 3 The growth stage wise growing degree days requirement of Indian potato varieties

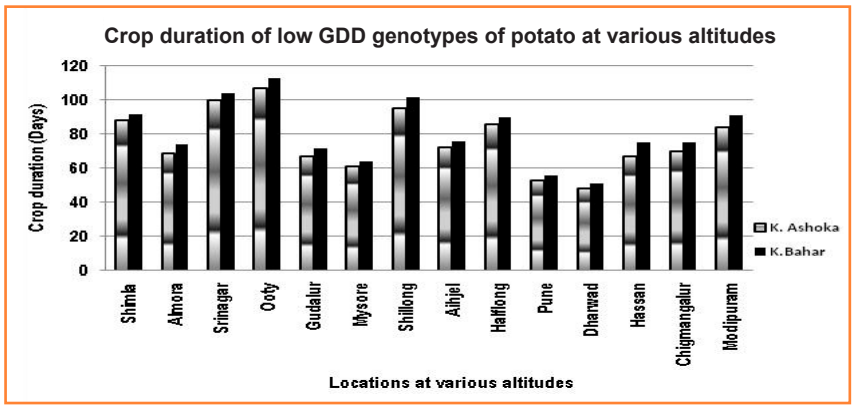

Figure 4 The location wise variation on varietal duration based on their growing degree days accumulation for low GDD potato cultivars K. Ashoka and K. Bahar

Accordingly, the varietal crop duration calculated for different altitudes based on the GDD accumulation indicated that there is a huge variation in varietal durations of the same variety at different locations. Accordingly, the stage wise GDD requirement of 10 Indian potato varieties showed varietal specificity (Singh et al., 2003). The GDD requirement from planting to germination ranged from 240 Cd (K. Bahar) to 325 Cd (K. Lalima and K. Sinduri) with varieties mean value of 296.5 Cd. Similarly, the GDD required for germination to stolon formation ranged from 230 Cd (K. Bahar) to

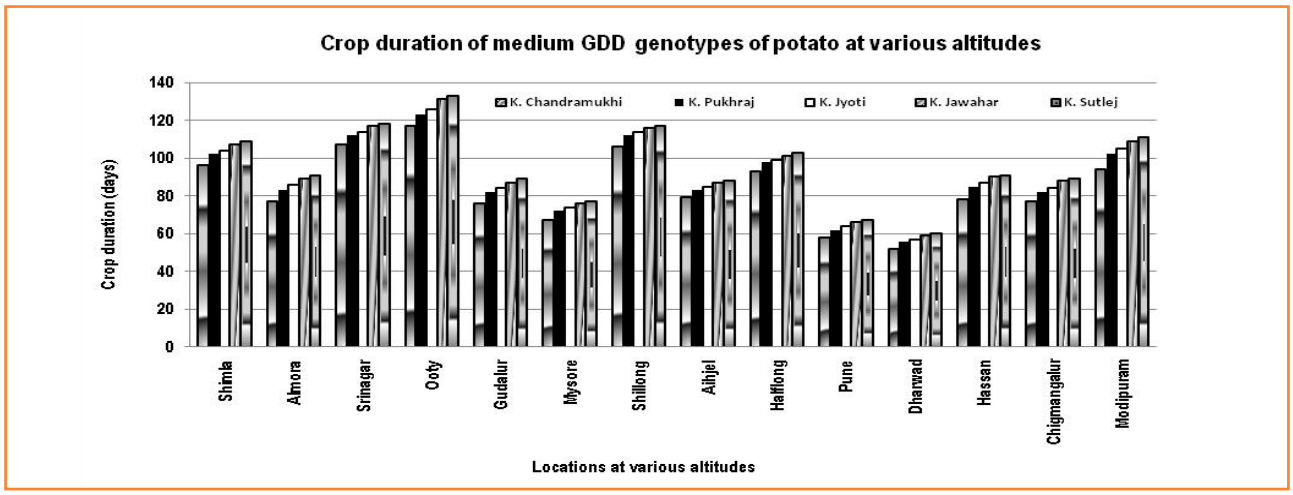

Figure 5 The location wise variation on varietal duration based on their growing degree days accumulation for medium GDD potato cultivars K. Chandramukhi, K. Pukhraj, K. Jyoti, K. Jawahar and K. Sutlej

440 Cd (K. Sinduri) with varietal mean value of 326 Cd. For stolon formation to tuber maturity, it ranged from 650 Cd (K. Asokha) to 950 Cd (K. Sinduri) with mean value of 814 Cd. Hence, the total GDD required for planting to tuber maturity ranged from 1,190 Cd (K. Asokha) to 1,715 Cd (K. Sinduri) with mean value of 1,436.5 Cd (Fig. 3). Based on GDD requirement, the varieties can be classified into three groups: as low, medium and long GDD varieties. The cultivar K. Ashoka and K. Bahar required comparitively lesser GDD (<1,300 Cd), K. Chandramauki, K. Pukhraj, K. Jothi, K. Jawahar, K.Badshah and K.Satluj needed moderate level (1,300–1,600 Cd) and K. Sinduri required the highest level (>1,600 Cd). The low GDD required cultivar K. Ashoka and K. Bahar completes its life cycle at 48 & 51days, respectively, at plateaus (Dharwad); however, they required 107 & 113 days, respectively, in high hills of SH (Ooty) due to their difference between heat unit accumulation (Fig. 4). The similar trend was observed for medium and long GDD varietes too, as K. Chandramuki (117), K. Pukhraj (123), K. Jothi (126), K. Jawahar (133) and K. Sutlej (136) required longer days at high hills of SH (Ooty) as compared to plateaus (Dharwad) (52, 56, 57, 59 and 60 days, respectively) (Fig. 5). However, these varieties needed only 94, 102, 105, 109, 111 days, respectively, at plains location (Modipuram). The long GDD required genotypes such as K Badshah, K. Lalima and K. Sinduri needed 60, 62 and 70 days at plateaus as compared to 136 and 139 days, respectively, whereas K Sinduri could not recieve sufficient GDD at high hills of SH (Ooty) as the suitable growing windows were not sufficient, hence it should be harvested as premature. This finding is supported by the results of rice crop duration which was found reduced under elevated temperature (>4 °C of normal) to 12 days earlier (96 days), and at >2 °C had 6 days earlier (102 days) as compared to that of ambient temperature (108 days). Similarly, the elevated temperature of >4 °C (1,641 °Cd) showed lower accumulated heat units for attaining active tillering to harvest than elevated temperature of 2 °C (1,583 °Cd) and ambient temperature (1,523 °Cd) (Arti Rani and Maragatham, 2013). Hence, it is concluded from the above facts that *kharif* growing locations required comparatively later maturing varieties for hills and plateaus (excepting Pune and Dharwad) compared to the plains.

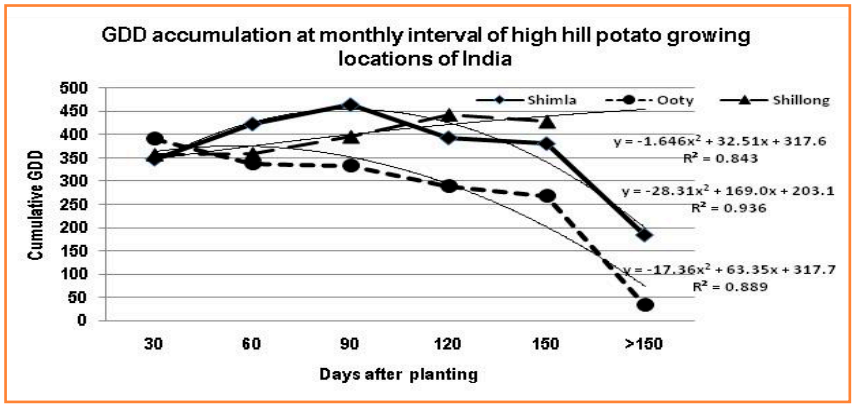

Figure 6 The mean monthly GDD accumulations and their progressive difference over high hill locations of India

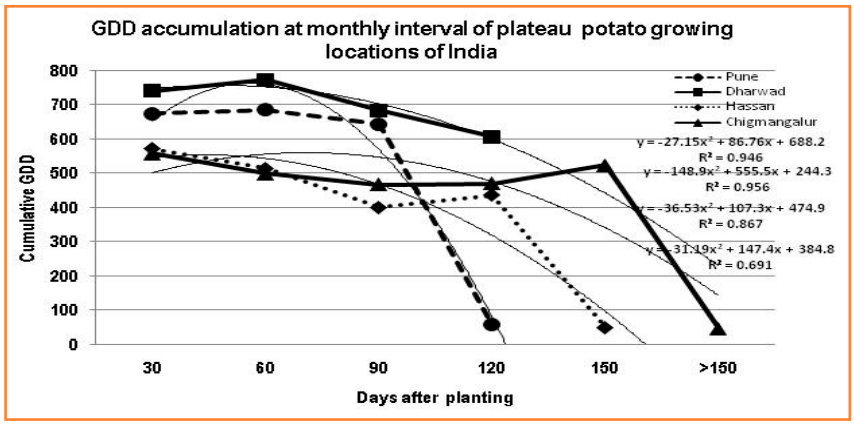

Figure 7 The mean monthly GDD accumulations and their progressive difference over plateau locations of India

Varietal selection

Although most of the potato varieties reported to require a minimum of 70–90 days of favourable cool season to obtain an economical tuber yield for ware purpose, the longer favourable season up to 110–120 days along with enough accumulated heat units results in substantially higher returns, provided the growth stage wise varietal GDD demand is fulfilled. In order to understand, the GDD accumulated at monthly interval for high hills and plateau representative location was compared with stage wise GDD demand of the cultivars. The Indian genotypes demands GDD for germination (296.5 Cd), stolonisation (326.0 Cd) and tuber maturity stages (814.0 Cd) revealed that the required GDD was accumulated at 30 DAP for germination and 60 DAP for stolonisation, irrespective of hills. However, from stolonisation to tuber maturity it demanded higher GDD, which is fulfilled only at NEH and NH (>350 Cd). On the other hand, in SH the GDD was descending <350 Cd (Fig. 6), which necessitates greater radiation use efficient cultivars for SH for achieving higher yield. On the contrary, the growth stage wise GDD accumulated in plateaus were exorbitantly higher than the GDD required for Indian genotypes (Fig. 7); hence the selection or developing new early matuting cultivars with greater radiation use efficiency is further enhancing the yield.

Environment exploitation for higher tuber yield

A little yield progress has been achieved in mid-early and late potato cultivars (Schuster, 1997) of German federal

varieties (Douches et al., 1996) and U.S. potato cultivars for a period of three decades which strongly explained the lack of yield improvement in early maturity genotypes of the most modern cultivars. In India, too, the coefficient of variation on area and production variability analysed for decade period revealed greater yield variability from high yield to high variability and low yield to low variability among potato growing states (Saxena and Mathur, 2013). The lower yield coupling with low/high yield variability states are the areas where unfavourable weather prevails with biotic and abiotic stress pressure and lack of ideal varieties resulting in their low yields (Sananse, Borude and Patil, 1990). Hence, modifying phonological patterns of crop is a prime requisite to avoid or minimize stress (Lopes and Reynolds, 2010) as efficient management on phonological basis is determined by Incident solar radiation (Monteith and Unsworth, 1990), radiation use efficiency (Mitchell, Sheehy and Woodward, 1998; Sinclair and Muchow, 1999) and harvest index (Belanger et al., 2001).

Long back, Donald (1968) designed a plant ideotype for small grain cereal having a short stem, a few erect leaves, a high harvest index and erect an ear and a single culm. The two hundred years of modern breeding of potato has resulted in a great diversity of modern lines; however, genotypic variation for photosynthetic traits is still lacking (Street et al., 2009) and reflects the necessity of ideotype breeding. The modern potatoes have a high harvest index of around 0.80 and potential yields of 140 $t.ha^{-1}$ (Mackay, 1996: Beukema and van der Waag, 1990) depending upon the length of growing season and temperatures. In tropics and sub-tropics, the possibility to reach the level of yields obtained in temperate zone is remote due to its shorter growing season and a lower efficiency per unit of intercepted radiation. However, *kharif* potato has a greater scope for yield exploitation through enhancing radiation use efficiency and light interception characteristics as it is grown under long day conditions with long growing period. Hence, the transition from qualitative to quantitative and from morphological to phonological traits in designing crop ideotype is essential to nullify the environment effect (Hunt, 1993) through species selection to plant architecture modification.

Species selection

Prioritising the plains, the Indian potato breeding program is focused mainly on Species *Solanum andigena* due to its ability to tuberisation under short day conditions as against *S. tuberosum* (long day conditions). Hence, *Tuberosum* (female) × short-day *Andigena* hybrids were used for the sub-tropic plains of India, these hybrids showed a yield advantage of 19% over *Tuberosum* × *Tuberosum* hybrids in the second clonal generation at harvest 120 days after planting (Gopal, Chahal and Minocha, 2000) although *Andigena* parents were lower yielding as compared to *Tuberosum* (6.02 versus 10.78 $kg.plot^{-1}$). As a result, a number of Indian potato cultivars have been developed from *Tuberosum* × *Andigena* crosses, including K. Pukhraj, K. Giriraj, K. Chipsona-2 and K. Shailja (Kumar et al., 2008). However during the same period in sixties, the *long-day* adapted *Andigena* (*Neotuberosum*) potatoes parents were used in European and North American breeding programmes (Glendinning, 1975) for long day conditions. The *Tuberosum* × *long-day Andigena* hybrids have shown yield advantages of 7 to 21% over *Tuberosum* × *Tuberosum* hybrids. Mass selection method adopted to produce a population of *S. phureja*/*S. stenotomum* for *long-day* North European conditions (Carroll, 1982), and achieved average 26% higher yielding hybrids than five *Tuberosum* cultivars (Pentland Crown, Desiree, Maris Piper, Pentland Dell and Record) (Carroll and De Maine, 1989). Cubillos and Plastid (1976) found that under short days in Mexico, Colombia and Peru, *long-day*-adapted (*Andigena* × *Andigena*) hybrids outyielded *Tuberosum* × *Tuberosum* hybrids and in Colombia they also outyielded their *Tuberosum*×*long-day Andigena* hybrids. Hence, breeding for *kharif* potato needed *Neotuberosum*, *S. phureja*/*S. stenotomum* parents based offspring to attain yield rather than *S. tuberosum* × *S. andigena* (short day adapted) based offsprings.

Plant architecture

Plant architecture is a three-dimensional organization of a part of a plant, encompassing branching pattern, plant height, leaves arrangement and the structure of reproductive organs which determines its adaptability to cultivation, harvest index and potential yield (Reinhardt and Kuhlemeier, 2002). Under *kharif* season, plant height had significantly positive correlation with number of leaves per plant, and in turn, the number of leaves had positive and significant correlation with days to tuber initiation, days to tuber maturity and tuber weight (Amadi, Ene-Obong and Okoc, 2008) justifies the delay in tuber initiation perhaps allowing for optimal foliage development before tuber initiation phase, and consequently delay in maturity allows for a longer period for the storage of assimilates, implying the taller plant with late maturity group suits for higher hill regions. A significantly negative correlation between tuber yield and days to tuber initiation; and days to maturity suggested that an early bulking coupled with a sustained partitioning of assimilates to tubers invariably lead to higher yield (Amadi and Ene-Obong, 2007) indicating early bulking genotypes suits invariably for mid and low hill regions. Fekadu, Petros and Zelleke (2013) found that tuber yield had positive correlation with plant height, biological yield, harvest index and big tuber percentage, while and negatively correlating with small and medium tuber percentage at both the phenotypic and genotypic levels. The greater genetic variability in potato for plant height (189 to 728 mm), plant spread (146 to 542 mm) and leaf area (88.6 to 217.5 cm^2) have higher phenotypic coefficient value than the genotypic value (Regassa and Basavaraj, 2005) and these traits had moderate heritability coupled with low genetic advance indicating that these traits can be improved by the hybridization method.

Fine-tuning the vegetative-to-reproductive growth balance is a way to manipulate varieties showing different growth habits (Spielmeyer, Ellis and Chandler, 2002). In potato, the vegetative peak reaches at about 60 days of planting (source) supplies sink to the tuber which start initiating at 35 days of growth. Tuber initiation involves a shift from stolon elongation to radial swelling of the sub-apical region, accompanied by a decline of alkaline and

acidic invertase, and an increase of sucrose synthase and fructokinase activity (Ross et al., 1994). The rise in sucrose synthase and fructokinase activities is positively correlated with the onset of starch and storage protein biosynthesis (Ross et al., 1994). Hence, the first formed tubers are larger in size due to dominance sink causing poorly developed tuber in latter forming stolon due to sink strength in tubers. In order to prolong and uniform tuber size, the ensured and balanced source and sink relation is maintained in semi-determinate growth genotypes. In similar line, stem characters and stem diameter exhibiting poly genes with additive genetic effect had highly positive correlation with 1,000-KW (kernal weight) and GYPS (grain yield per stem) in wheat genotypes. Hence, semi-determinate with erect stem genotype would yield better by manipulation in sink and source relation in potato for *kharif* environments.

Leaf morphology and its orientation

Dickinson, Parker and Strauss (1987) reported that differences in leaf shape are often more inheritable and independent on the environment. The genetic variability for leaf morphology (Hue, Chandran and Boyce, 2010), and the larger leaf surface area is associated with increasing light absorption. Genetic variation reported for leaf number per plant ranged from 30.3 (Granola) to 93.3 (Agria) and it is genotype-specific (Ozturk and Yildrim, 2014). The presence of complex edges and lobes in larger leaves enables to disperse the absorbed heat very rapidly, and waxy surface in younger leaves prevents or minimise the transpiration rate as they locate at the top of the plant (C.I.P., 2008). In many species, vein density has been correlated with hydraulic conductivity of water and maximum photosynthetic rate in leaves (Brodribb, Field and Jordan, 2007) and the smaller leaf area has been associated with greater vein density that may contribute to increased abiotic stress tolerance (Scoffoni et al., 2011). Irrespective of shape and size of leaf, Leaf Area index (LAI) has been defined as the area of green leaves per unit area of the ground (Jonckeere, 2004), a higher and lower value of LAI indicating a denser and sparse crop canopy, respectively (Boken and Chandra, 2012). LAI is a stage of genotype and its growing condition specific (Lopes et al., 2013). In potato, LAI ranged from 1.40 (Dakchip) to 6.60 (Pungo), where the maximum LAI recorded at 61 DAP (Monalisa) (Nunes et al., 2006). However, the cv. Atlantic, Chipbelle and DTO-33 showed no decline in their LAI up to 73 days of planting (DAP) indicating a better abiotic stress tolerance response. Leaf area expansion is a determinant of crop growth rate (Goudriaan and van Laar, 1994); it increases with LAI particularly at early growth stages, because the relative increases in the interception of photosynthetically active radiation (IPAR) are largest when leaf is small (Jamieson et al., 1998). Potato genotypes with warmer crop canopies under irrigated conditions are less susceptible to drought than genotypes with cooler canopies (Stark, Pavek and McCann, 1991) due to the rate of transpiration driven cooling of the leaves. Genetic manipulation of leaf angle is not complex, and is thought to be controlled by only two to three genes. In Dasiree, the dry matter distribution pattern that diverted substantially and tuber filling started earlier than the cultivars of same maturity group, but during tuber filling a greater proportion of the assimilate diverted in the leaf growth (Spitter and Schapendock, 1990) indicates the suitability of erectophile leaf with medium to late bulking genotypes for *kharif* season where later growth stage seldom faces moisture stress in the soil due to adequate rainfall.

Leaf morphology determines leaf photosynthetic rate which can be improved by breeding (Crosbie, Pearce and Mock, 1981) and progress could be achieved for high photosynthetic CO_2 exchange rate (Mahon and Hobbs, 1981). The canopy photosynthesis on ground cover basis was ranged from 1.72 to 4.34 g $CO_2.m^{-2}.hr^{-1}$ (Bhagsari, 1988), cv. Pungo had higher values than other genotypes. The mean adaxial and abaxial stomatal conductance was 0.86 and 1.46 $cm.sec^{-1}$, respecively. Dry matter partitioning to tubers ranged from 8.9% (Pungo) to 55.5% (Atlantic) 67 DAP and the tuber yield ranged from 9.6 to 27.8 $MT.ha^{-1}$, indicating the suitability of cultivar Atlantic for growing in a warm climate. Cieply (1976) concluded that assimilation rates can be used as a physiological criterion for rapid selection in potato breeding. Measured rates of CO_2 uptake by 18 clones of potato under standard conditions (Mol and Henniger, 1978) and the stomatal number per leaf at upper surface ranged from 4 (A6948) to 50 (A66107-51). The area of stomatal apparatus was also found to range from 0.1 to 2.2 at upper surface and 6.8 and 9.6 at lower surface of respective potato clones. However, the CO_2 uptake was found higher in clone A-6948 (9.2) as compared to A-66107-51(7.6 mg CO_2 $mg^{-1}.chl.h^{-1}$), giving a surprising contribution to total carbon assimilation that Lemhi has an unusually high rate of CO_2 assimilation through the upper leaf surface, and A6948-4 an unusually high rate through the lower leaf surface; through breeding, these two characters can be combined for the high carbon assimilation rates of Lemhi's upper leaf surface and A6948-4's lower leaf surface. The greater leaf carbon exchange rate of Dara-5 transgenic plants did not result in increased yields which is explained by poor C-use efficiency, low sink strength of the tubers, or both (Schittenhelm, Sourell and Löpmeier, 2006). The transgenic plants exhibited relatively higher investment of biomass into photosynthetic leaf area, stems, and roots and less to tubers than the nontransgenic plants due to the differing rate of dark respiration among the parts (Vose and Ryan, 2002) because tubers have lower respiration rates than other potato plant parts, especially leaves (Winkler, 1971).

Root architecture and drought tolerance

Manpulation of root architecture is imperative for *kharif* potato as it frequently encounters nutritional deficiency and drought stress. In general, potato cultivars are shallow rooted and often produce most of their roots in the plough layer (Iwama, 2008; Iwama and Yamaguchi, 2006). However, an inhibition of root growth by high soil strength greater than 1 MPa under rainfed situation (Miller and Martin, 1987) results in low harvest index as against an irrigated crop with high harvest index (Vos and Haverkort, 2007). A wider variation for root traits in European tetraploid potato (*S. tuberosum* Group *Tuberosum*), diploid *Phureja* potatoes (*S. tuberosum* Group *Phureja*) and *Neotuberosum* lines (selected from *S. tuberosum* Group *Andigena*) has been observed (Wishart et al., 2009). The total root

length per plant varied from 0.38 m (*Tuberosum* variety Pentland Dell) to >100 m (*Phureja* variety Mayan Twilight). *Phureja* line (Mayan Gold) had the longest and thinnest roots. The number of stolon roots and basal roots amongst the cultivars varied, the *Phureja* line, Mayan Gold, had significantly more of both root classes, their relative proportions too, suggesting potential genetic difference in resource partitioning. A positive correlation between root pulling resistance and tuber yield (Wall et al., 2006) and root mass correlates well with leaf mass, and tuber yield (Deguchi et al., 2010) observed earlier simplifies overcoming difficulty in root studies in potato. Most N efficient hybrid JX-576 had significantly higher mean root length, root surface area, and root volume than least N efficient cultivar Kufri Jyothi (Trehan and Singh, 2013). Average tuber yield reduction per mm water deficit has been estimated at 117 kg.ha^{-1} (Vos and Groenwold, 1988) and a decrease of leaf water potential from -0.5 to -0.9 MPa reduced photosynthesis by 58% and it decreased the internal CO_2 concentration by 29% (Vos and Oyarzun, 1987). The CO_2 concentration and assimilation are positively correlated and a 10% increase in tuber yield is estimated for every 100 ppm increase in CO_2 concentration (Miglietta et al., 1998). The simulation study of 16 potato growing locations also indicated that locations viz., Chitoor and Kolar appear to be ideal for screening studies as the night temperature at these locations is between 20 and 22 °C for most of the crop season and varieties with heat tolerance greater than K. Surya can be selected at these locations (Minhas et al., 2011). K. Surya has been identified for successful growing of potato for these areas where at least 70 days are available with night temperatures ranging from 18 to 22 °C. Thus, it is suitable for most parts of peninsular and coastal India e.g., in the Andaman and Nicobar islands the variety gave a yield of 14 t.ha^{-1} (Minhas et al., 2011).

Hypothetical attainable yield estimation through ideotype breeding approach for *kharif* areas.

In addition to the yield determinant traits, the available length of the growing season also determines the yield (Haverkort, 1990). The high hills had suitable temperatures for longer duration and longer rainfall season necessitates the demands for late variety which should utilise the long growing season for better yields as compared to early varieties. The low GDD varieties (K. Ashoka and K. Bahar) comes to harvest very early at high hills (86–113 days), plateaus (48–75 days) and plains (84–91 days), revealing the more unutilised suitable growing period due to senescence of present day varieites. The medium GDD varieties such as K Chandramuki, K. Pukhraj, K. Jothi, K Jawahar and K. Sutlej also complete growth cycle within 96–133 at high hills, 76–88 at mid hills, 67–118 days at low hills, 52–91 days at plateaus and 94–111 days in the plains reflecting more unutilised suitable growing period. Similar trend has been observed for high GDD varieties such as K Badshah, K. Lalima and K. Sinduri across growing locations.

It is clear from the fact that the crop duration should be effectively utilised by developing late maturing varieties as compared to the present day varieties. Hence, an ideotype have stay green traits reflects the maintenance of photosynthetic activity longer than a capacity for light harvesting during the mobilisation of carbon produce to the harvested organs (Yan, 2004), can be exploited as timing of senescence is a heritable and manipulated (Shahnazari, 2008) in potato. Genotypes that are late maturing have full ground cover with green foliage till the end of the available growing season; however, it is an indication of unfavourable distribution of dry matter to the foliage rather than to the harvestable parts. This should be managed by developing ideotype with semideterminant growth habit, which balances the vegetative as well as reproductive parts.

A hypothetical attainable yield was estimated for different *kharif* growing regions of India having with existing growing period the changing

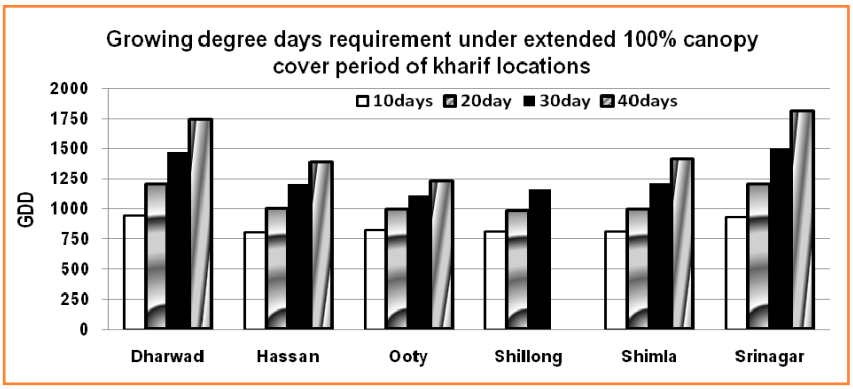

Figure 8 Growing degree days accumulation at extended 100% canopy cover for 40 days at various *kharif* potato growing locations

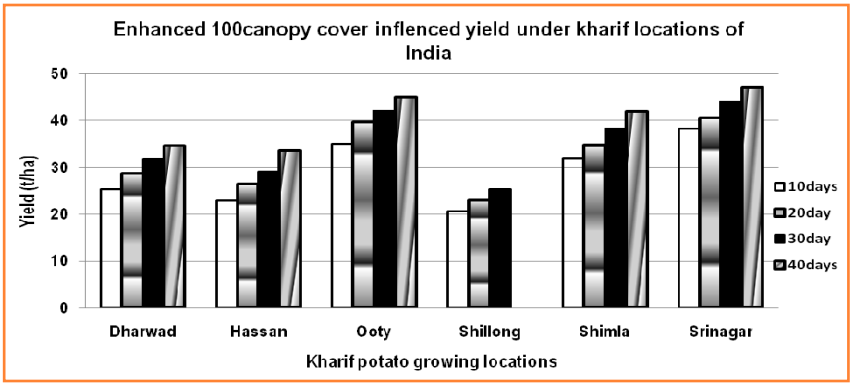

Figure 9 The predicted yield variation for extended 100% canopy cover for 40days at various *kharif* potato growing regions

canopy cover (100%) duration extended by 10, 20, 30 and 40 days. By increasing canopy cover (100%) to 40 days, the GDD accumulation during the growing season could be enhanced upto 800 Cd (Dharwad), 585 Cd (Hassan et al., 2007), 406 Cd (Ooty), 602 Cd (Shimla) and 886 Cd (Srinagar) additionally in different *kharif* areas. Under the condition of harvesting of the additional heat units and converting in terms of drymatter, the attainable yield of 25.4, 28.7, 31.7 and 34.7 $t.ha^{-1}$, respectively, could be obtained at Dharwad. Similarly, the values of 31.9, 34.9, 38.3 and 42.0 $t.ha^{-1}$ were indicated for Shimla (Fig. 8).

It has also resulted from the fact that despite higher heat unit accumulation in Dharwad and Srinagar, the attainable yield could reach up to 34.7 and 47.1 $t.ha^{-1}$, respectively (up to 30 days only). However, the locations Ooty and Shimla register higher values for attainable yield (45.1 and 42.0 $t.ha^{-1}$, respectively) despite the low level of heat accumulation (<1,500 °Cd). In Shillong, the 100% canopy cover cannot be expanded for 40 days of its attainment due to the lack of suitable growing period for potato (<1,250 °Cd) (Fig. 9).

Way Forward

Presently, *kharif* potato is complexly a long day crop grown mainly rainfed in hills and plateaus, and affected by high temperature during rainy season in plains; the area under *kharif* potatoes has not extended to plains. Developing an ideal phenotype having traits introgressed using physiological breeding approach for enhanced radiation and light use efficiency under longday/day neutral background is imperative. The yield in potato under stress free conditions is determined by the canopy cover, radiation use efficiency and partitioning ratio of genotypes. The amount of total radiation intercepted by green active foliage depends on the amount of solar radiation and on the proportion that intercepted (based on the Leaf area index, leaf angle and scattering nature of leaf). The size and shape of the leaves with complex edges are associated with stress tolerance and dispersion of absorbed heat is a very desirable trait in heat stressed environments. The vein density has been correlated with hydraulic conductivity of water and maximum photosynthetic rate in leaves (Brodribb, Field and Jordan, 2007) and enhanced water use efficiency.

Improvement in root traits like root depth and root length density is important for developing cultivars for rainfed condition. Wider variation in rooting traits of a range of potato genotypes including the European tetraploid potato, diploid and *Neotuberosum* lines showed the total root length per plant varied from 38 m (*Tuberosum* variety Pentland Dell) to >100m (*Phureja* variety Mayan Twilight) (Wishart et al., 2009). A positive correlation found between root pulling resistance and tuber yield, root mass correlates well with leaf mass and tuber yield (Deguchi et al., 2010) could be used as an indices for selecting ideal parental line for *kharif* potato breeding program. Hence, a phenotype having semi determinate growth type with erectophile lobed leaf margins of waxy surface having dense venation at arial and higher root diameter with higher tuber surface roots at underground coupling of higher radiation and light use efficiency is ideal for *kharif* potatoes.

References

AMADI, C.O. – ENE-OBONG, E.E. 2007. Genetic variability and inter-relationships of some potato attributes in Jos Plateau, Nigeria. In Nigerian Journal of Botany, vol. 20, 2007, no. 1, pp. 233–245.

AMADI, C.O. – ENE-OBONG, E. E. – OKOC, B. E. 2008. Path analysis of yield of some potato hybrids and their progenitors in Northern Guinea Savanna of Nigeria. In PAT, vol. 4, 2008, no. 2, pp. 28–37.

ARTHI RANI, B. – MARAGATHAM, N. 2013. Effect of elevated temperature on rice phenology and yield. In Indian Journal of Science and Technology, vol. 6, 2013, no. 8, pp. 5096–5098.

BELANGER, G. – WALSH, J.R. – RICHARDS, J.E. – MILBURN, P.H. – ZIADI, N. 2001. Tuber growth and biomass partitioning of two potato cultivars grown under different N fertilization rates with and without irrigation. In American Journal of Potato Research, vol. 78, 2001, pp. 109–117.

BEUKEMA, H.P. – VAN DER WAAG, D.E. 1990. Introduction to potato production. Wageningen : Pudoc, 1990, pp. 23–24.

BHAGSARI, A.S. 1988. Photosynthesis and stomatal conductance of selected root crops as related to leaf age. In Crop Science, vol. 28, 1988, pp. 902–906.

BOKEN, V.K. – CHANDRA, S. 2012. Estimating leaf area index for an arid region using spectral data. In African Crop Science Journal, vol. 20, 2012, no. 4, pp. 215–223.

BRADSHAW, J.E. 2009. A Genetic perspective on yield plateau in potato. In Potato J., vol. 36, 2009, no. 3–4, pp. 79–94.

BRODRIBB, T.J. – FIELD, T.S. – JORDAN, G.J. 2007. Leaf maximum photosynthetic rate and venation are linked by hydraulics. In Plant Physiology, vol. 144, 2007, pp. 1890–1898.

CIEPLY, J. 1976. The productivity of photosynthesis of several varieties of spring barley and potatoes as an index of their fertility (Abstr.). Krakow : RepAcadAgric, 1976.

C.I.P. 2008. Inte rnational Potato Center (ICP). 2008. www.cipotato.org/sweetpotato/

CARROLL, C.P. 1982. A mass-selection method for the acclimatization and improvement of edible diploid potatoes in the United Kingdom. In Journal of Agricultural Science Cambridge, vol. 99, 1982, pp. 631–640.

CARROLL, C.P. – DE MAINE, M.J. 1989. The agronomic value of tetraploid F_1 hybrids between potatoes of group *Tuberosum* and group *Phureja*/Stenotomum. In Potato Research, vol. 32, 1989, pp. 447–56.

CPRI. 2011. Vision 2030. Central Potato Research Institute, Shimla, India, 2011, 40 p.

CROSBIE, T.M. – PEARCE, R.B. – MOCK, J.J. 1981. Recurrent phenotypic selection for high and low photosynthesis in two maize populations. In Crop Science, vol. 21, 1981, pp. 736–740.

CUBILLOS, A.G. – PLAISTED, R.L. 1976. Heterosis for yield in hybrids between *S. tuberosum* ssp. *Tuberosum* and *tuberosum* ssp. *andigena*. In American Potato Journal, vol. 53, 1976, pp. 143–150.

DEGUCHI, T. – NAYA, T. – WANGCHUK, P. – ITOH, E. – MASSUMOTO, M. – ZHENG, X. – GOPAL, J. – IWAMA, K. 2010. Aboveground characteristics, yield potential and drought tolerance in "Konyu" potato cultivars with large root mass. In Potato Research, vol. 53, 2010, pp. 331–340.

DICKINSON, T.A. – PARKER, W.H. – STRAUSS, R.E. 1987. Another approach to Leaf Shape Comparisons. In Taxonomy, vol. 36, 1987, no. 1, pp. 1–20.

DONALD, C.M. 1968. The breeding of crop ideotype. In Euphytica, vol. 17, 1968, pp. 385–403.

DOUCHES, D.S. – MAAS, D. – JASTRZEBSKI, K. – CHASE, R.W. 1996. Assessment of potato breeding progress in the USA over the last century. In Crop Science, vol. 36, 1996, pp. 1544–1552.

FAO. 2014. Statistical databases FAOSTAT. http://faostat.fao.org/site/567/default. aspx#ancor, 2014.

FEKADU, A. – PETROS, Y. – ZELLEKE, H. 2013. Genetic variability and association between agronomic characters in some potato (*Solanum tuberosum* L.) genotypes in SNNPRS, Ethiopia. In International Journal of Biodiversity and Conservation, vol. 5, 2013, no. 8, pp. 523–528.

GLENDINNING, D.R. 1975. Neo-*Tuberosum*: New potato breeding material. 2. A comparison of Neo-*Tuberosum* with unselected *Andigena* and with *Tuberosum*. In Potato Research,vol. 18, 1975, pp. 343–350.

GOPAL, J. – CHAHAL, G.S. – MINOCHA, J.L. 2000. Progeny mean, heterosis and heterobeltiosis in *Solanum tuberosum* × *tuberosum* and *S. tuberosum* × *andigena* families under a short day sub-tropic environment. In Potato Research, vol. 43, 2000, pp. 61–70.

GOUDRIAAN, J. – VAN LAAR, H.H. 1994. Modelling potential crop growth processes. Dordrecht : Kluwer Academic Publishers, 1994.

HASSAN, Q.K. – CHARLES, P. – BOURQUE, A. – MENG, F.R. – RICHARDS, W. 2007. Spatial mapping of growing degree days: an application of MODIS-based surface temperatures and enhanced vegetation index. In Journal of Applied Remote Sensing, vol. 1, 2007, pp. 1–12.

HAVERKORT, A. J. 1990. Ecology of potato cropping systems in relation to latitude and altitude. In Agricultural Systems, vol. 32, 1990, no. 3, pp. 251–272.

HAVERKORT, A.J. – FRANKE, A. C. – STEYN, J. M. – PRONK, A. A. – CALDIZ, D. O. – KOOMAN, P. L. 2015. A Robust Potato Model: LINTUL-POTATO-DSS A. In J. Potato Research, 2015, no. 58, pp. 313–327.

HUE, S.M. – CHANDRAN, S. – BOYCE, A.N. 2010. ISHS Acta Horticulturae 943: Asia Pacific Symposium on Postharvest Research, Education and Extension 1 Variationsof Leaf and Storage Roots Morphology in *Ipomoea batatas* L. (*Sweet potato*) Cultivars. ISHS Acta Horticulturae. 2010, 943: Asia Pacific Symposium on Postharvest Research, Education and Extension.

HUNT, S. C. – HASSTEDT, S. J. – WU, L. L. – WILLIAMS, R. R. 1993. A gene-environment interaction between inferred kallikrein genotype and potassium. In Hypertension, vol. 22, 1993, no. 2, pp. 161–168.

IWAMA, K. 2008. Physiology of the potato: New insights into root system and repercussions for crop management. In PotatoResearch, vol. 51, 2008, pp. 333–353.

IWAMA, K. – YAMAGUCHI, J. 2006. Chapter 7. Abiotic Stresses. In Handbook of potato production, improvement, and postharvest management, ed. Gopal, J. – Khurana, S.M.P., New York : The Haworth Press, 2006, pp. 231–278.

JAMIESON, P.D. – SEMENOV, M.A. – BROOKING, I.R. – FRANCIS, G.S. 1998. Sirius: a mechanistic model of wheat response to environmental variation. In European Journal of Agronomy, vol. 8, 1998, pp. 161–179.

JONCKHEERE, I. – FLECK, S. – NACKERTS, K. – MUYS, B. – COPPIN, P. – BAREF, M. 2004. Review of methods for in situ leaf area index determination: theories, sensors and hemispheral photography. In Agricultural and Forest Meteorology, vol. 121, 2004, no. 1–2, pp. 19–35.

KUMAR, R. – KUMAR, V. – GOPAL, J. – LUTHRA, S.K. – PANDEY, S.K. 2008. Inventory of Potato Germplasm (Group *Andigena*) Collection. In Technical Bulletin, CPRI, Shimla, India, 2008, no. 86.

LOPES, M.S. – REYNOLDS, M.P. 2010. Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. In Functional Plant Biology, vol. 37, 2010, pp. 147–156.

LOPES, E.C. – JADOSKI, S.O. – SAITO, L.R. – SCHEIFITER DE RAMOS, M. 2013. Plant morphological characteristics and yield of Potato cv. Agata in function to fungicides application. In Brazilian Journal of Applied Technology for Agricultural Science, Guarapuava-PR, vol. 6, 2013, no. 1, pp. 37–46.

MACKAY, G.R. 1996. An Agenda for future potato research. In Potato Res, vol. 39, 1996, pp. 387–394.

MAHON, J.D. – HOBBS, S.L. 1981. Selection of peas for photosynthetic CO_2 exchange rate under field conditions. In Crop Science, vol. 21, 1981, pp. 616–621.

MC MASTER, G.S. – WILHELM, W.W. 1997. Growing degree-days: one equation, two interpretations. In Agricultural and Forest Meteorology, vol. 87, 1997, no. 4, pp. 291–300.

MILLER, D.E. – MARTIN, M.W. 1987. The effect of irrigation regime and subsoiling on yield and quality of three potato cultivars. In American Potato Journal, vol. 64, 1987, pp. 17–25.

MIGLIETTA, F. – MAGLIULO, V. – BINDI, M. – CERIO, L. – VACCARI, F.P. – LODUCA, V. – PERESSOTTI, A. 1998. Free air CO_2 enrichment of potato (*Solanum tuberosum* L.): development, growth and yield. In Global Change Biology, vol. 4, 1998, pp. 163–172.

MINHAS, J.S. – RAWAT, S. – GOVINDAKRISHNAN, P.M. – KUMAR, D. 2011. Possibilities of enhancing potato production in non-traditional areas. In Potato Journal, vol. 38, 2011, no. 1, pp. 14–27.

MITCHELL, P.L. – SHEEHY, J.E. – WOODWARD, F.I. 1998. Potential yields and the efficiency of radiation use in rice. IRRI Discussion Paper Ser. 32. Manila : IRRI, 1998.

MOL, L. – HENNIGER, A.W. 1978. Genotypische Photosynthescratevon Kartoffeln und ihre Mogliche RoUe fur die Ertagsbildung. In Photosynthetica, vol. 12, 1978, pp. 51–61.

MONTEITH, J.L. – UNSWORTH, M.H. 1990. Principles of environmental physics, 2nd ed., Oxford : Butterworth, 1990.

NUNES, J.C.S. – FONTES, P.C.R. – ARAUJO, E.F. – SEDIYAMA, C. 2006. Crescimento da batateira e absorcao de macronutrients influenciadospelossistemas de prepare de solo e irrigacao. In Pesquisa Agropecuaria Brasileira, vol. 41, 2006, no. 12, pp. 1787–1792.

OZTURK, G. – YILDIRIM, Z. 2014. Heritability estimates of some quantitative traits in potatoes. In Turkish Journal of Field Crops, vol. 19, 2014, no. 2, pp. 262–267.

PUSHKARNATH. 1976. Potato in Subtropics. New Delhi : Orient Longman, 1976, 289 p.

REGASSA, D. – BASAVARAJ, N. 2005. Genetic variability studies in potato (*Solanum tuberosum* L.). In Karnataka Journal of Agricultural Science, vol. 18, 2005, no. 1, pp. 87–90.

REINHARDT, D. – KUHLEMEIER, C. 2002. Plant architecture. In EMBO Report, vol. 3, 2002, pp. 846–851.

ROSS, H.A. – DAVIES, H.V. – BURCH, L.R. – VIOLA, R. – MCRAE, D. 1994. Developmental changes in carbohydrate content and sucrose degrading enzymes in tuberising stolons of potato (*Solanum tuberosum*). In Physiology Plantarum, vol. 90, 1994, pp. 748–756.

REINHARDT, D. – KUHLEMEIER, C. 2002. Plant architecture. In EMBO reports, vol. 3, 2002, no. 9, pp. 846–851.

SANANSE, S.L. – BORUDE, S.G. – PATIL, H.N. 1990. A study on variability and trends in area, production and productivity of rice in Konkan region of Maharashtra. In Journal of Maharashtra Agricultural University, vol. 15, 1990, no. 1, pp. 86–89.

SAXENA, R. – MATHUR, P. 2013. Analysis of potato production Performance and yield variability in India. In Potato J, vol. 40, 2013, no. 1, pp. 38–44.

SCHITTENHELM, S. – SOURELL, H. – LÖPMEIER, F.J. 2006. Drought resistance of potato cultivars with contrasting canopy architecture. In European Journal of Agronomy, vol. 24, 2006, pp. 193–202.

SCHUSTER, W.H. 1997. How much does plant breeding contribute to yield improvement of crops? In German Journal of Agronomy, vol. 1, 1997, pp. 9–18.

SCOFFONI, C. – RAWLS, M. – MCKOWN, A. – COCHARD, H. – SACK, L. 2011. Decline of leaf hydraulic conductance with dehydration: Relationship to leaf size and venation architecture. In Plant Physiology, vol. 156, 2011, pp. 832–843.

SCOTT, G.J. – SUAREZ, V. 2011. Growth rates for potato in India and their implications for industry. In Potato Journal, vol. 38, 2011, no. 2, pp. 100–12.

SINGH, B. – EZEKIEL, R. 2003. Influence of relative humidity on weight loss in potato tubers stored at high temperature. In Indian Journal of Plant Physiology, vol. 8, 2003, no. 2, pp. 141–144.

SHAHNAZARI, A. – AHMADI, S.H. – LAERKE, P.E. – LIU, F. – PLAUBORG, F. – JACOBSEN, S.E. – JENSEN, C.R. – ANDERSEN, M.N. 2008. Nitrogen dynamics in the soil-plant system under deficit and partial root-zone drying irrigation strategies in potatoes. In European Journal of Agronomy, vol. 25, 2008, pp. 65–73.

SINCLAIR, T.R. – MUCHOW, R.C. 1999. Radiation use efficiency. In Advances in Agronomy, vol. 65, 1999, pp. 215–265.

SPIELMEYER, W. – ELLIS, M.H. – CHANDLER, P.M. 2002. Semidwarf (sd-1), ´green revolution' rice, contains a defective gibberellin 20-oxidase gene. In Proceedings of National Acadomy of Science USA, vol. 99, 2002, pp. 9043-9048.

SPITTERS, C.J.T. – SCHAPENDONK, A.H.C.M. 1990. Evaluation of breeding strategies for drought tolerance in potato by means of crop growth simulation. In Plant and Soil, vol. 123, 1990, pp. 193–203.

STARK, J.C. – PAVEK, J.J. – MCCANN, I.R. 1991. Using canopy temperature measurements to evaluate drought tolerance of potato genotypes. In Journal of American Society of Horticultural Science, vol. 116, 1991, pp. 412–415.

STREET, K. – HAMILTON, R.S. – TAY, D. – TABA, S. – MACKAY, M. 2009. Mining germplasm banks for photosynthetic improvement-wheat, rice, potato, legumes and maize. In Applying photosynthesis research to improvement of food crops. (eds.) Gready, Jill E. – Dwyer, Simon A. – Evans, John R. Proceedings of a workshop held at the Australian National University, Canberra, Australian Capital Territory, Australia, 2–4 September 2009, pp. 112–129.

TREHAN, S.P. – SINGH, B.P. 2013. Nutrient efficiency of different crop species and potato varieties in Retrospect and prospect. In Potato Journal, vol. 40, 2013, no. 1, pp. 1–21.

VINCENTE, M.H. – ZSÖGÖN, A. – LOPODESÁ, A.F. – RIBEIRO, R.V. – PERES, L.E.E.P. 2015. Semi-determinate growth habit adjusts the vegetative-to-reproductive balance and increases productivity and water-use efficiency in tomato (*Solanum lycopersicum*). In Journal of Plant Physiology, vol. 177, 2015, pp. 11–19.

VOS, J. – GROENWOLD, J. 1988. Mean annual yield reductions of potatoes due to water deficits for Dutch weather conditions. In Acta Horticulturae, vol. 214, 1988, pp. 61–70.

VOS, J. – HAVERKORT, A.J. 2007. Water availability and potato crop performance. In Potato biology and biotechnology: Advances and perspectives. ed. Vreugdenhil, D. – Bradshaw, J. – GEBHARDT, C. – GOVERS, F. – TAYLOR, M. A. – MACKERRON, D.K.L. – ROSS, H. A. Amsterdam : Elsevier, 2007, pp. 333–351.

VOS, J. – OYARZUN, P.J. 1987. Photosynthesis and stomatal conductance of potato leaves – effects of leaf age, irradiance and leaf water potential. In Photosynthesis Research, vol. 11, 1987, pp. 253–264.

VOSE, J.M. – RYAN, M.G. 2002. Seasonal respiration of foliage, fine roots, and woody tissues in relation to growth, tissue N, and photosynthesis. In Global Change Biology, vol. 8, 2002, pp. 182–193.

WALL, G.W. – GARCIA, R.L. – KIMBALL, B.A. – HUNSAKER, D.J. – PINTER, P.J. JR. – LONG, S.P. – OSBORNE, C.P. – HENDRIX, D.L. – WECHSUNG, F. – WECHSUNG, G. – LEAVITT, S.W. – LAMORTE, R.L. – IDSO, S.B. 2006. Interactive effects of elevated carbon dioxide and drought on wheat. In Agronomy Journal, vol. 98, 2006, pp. 354–381.

WISHART, J.T.S. – GEORGE, L.K. – BROWN, J.A. – THOMPSON, G. – RAMSAY, J.E. – BRADSHAW, P.J. – WHITE, P.J. – GREGORY. 2009. Variation in rooting habit of potatoes: potential for improving resource capture. In International Symposium "Root Research and Applications, RootRAP, Boku – Vienna, Austria, 2–4 September 2009, pp.1–4.

WINKLER, E. 1971. Potato cultivation in Tyrol. II. Photosynthetic efficiency and respiration in different potato varieties. In Potato Research, vol. 14, 1971, pp. 1–18.

YAN, H. – KANG, M. – DE REFFYE, P. – DINGKUHN, M. 2004.A dynamic, architectural plant model simulating resource-dependent growth. In Annals of Botany, vol. 93, 2004, pp. 591–602.

YOU, L. – ROSEGRANT, M.W. – WOOD, S. – SUN, D. 2009. Impact of growing season temperature on wheat productivity in China. In Agricultural Meteorology, vol. 149, 2009, pp. 1009–1014.

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GENETIC ANALYSIS OF SOME AGRONOMIC TRAITS IN OKRA (*ABELMOSCHUS ESCULENTUS* L. [MOENCH])

Muyideen Oluseyi OLAYIWOLA[2]*, Deborah Doyinsola OLANIRAN[1], Adesola Lateef NASSIR[2], Omolayo Johnson Ariyo[1, 2]

[1]Olabisi Onabanjo University, Ago-Iwoye, Nigeria
[2]Federal University of Agriculture Abeokuta, Nigeria

A study was carried out at the Federal University of Agriculture Abeokuta, Nigeria to determine the gene action underlying the inheritance of important agronomic traits as well as the general combining ability (GCA) and specific combining ability (SCA) of the parents and hybrids, respectively. Ten hybrids were developed by crossing five lines to two testers. The hybrids and parents were evaluated on the field in a randomised complete block design replicated three times, and data were collected on days to 50% flowering, number of branches, stem diameter, plant height, pod length, pod width, pod weight, number of pods and pod yield. The data were subjected to line by tester analysis and results showed substantial variability among the genotypes for some of the characters measured. Days to 50% flowering, number of pods and pod yield were largely under additive gene action while non-additive gene action was more important in the inheritance of plant height. Favourable GCA and SCA effects for days to 50% flowering were observed in NGB00356, NGB00326 and NGB00347 × NGB00326, respectively. The tester NGB00326 had a positive and significant GCA effect for number of pods while the highest positive SCA effect for pod yield was found in NGB00297 × NGB00326. Thus, NGB00356 and NGB00326 could be considered as sources of alleles for development of early maturing while the cross NGB00297 × NGB00326 could be exploited for high yielding okra genotypes.

Keywords: earliness, high yielding, hybrids, okra

Okra (*Abelmoschus esculentus* L. [Moench]) is an important vegetable crop in West and Central Africa (Nwangburuka et al., 2012), where it is referred to as "a perfect villager's vegetable" due to the high dietary fibre and seed protein balance it offers in diets (Kumar et al., 2010). However, low yields have been recorded on farmers' fields due to the lack of adapted genotypes, narrow genetic base of existing cultivars and unpredictable length of the growing season (Ahiakpa et al., 2013). It is vital to develop okra varieties with early maturing and high yielding potentials. Arora et al. (2007) noted that these traits were polygenic and their expression greatly influenced by the environmental fluctuations. They submitted that an understanding of the gene action that controls the inheritance of fruit yield and earliness related traits in okra was important.

Line × Tester analysis proposed by Kempthorne (1957) is a popular mating design among plant breeders and its efficiency in detecting the nature and magnitude of gene action controlling important agronomic traits in crops has been reported (Prakash et al., 2002). Furthermore, line × tester analysis provides estimates of specific combining ability (SCA) of each cross and general combining ability (GCA) of lines and testers and at the same time, it is helpful in estimating various types of gene actions that are important in the expression of quantitative traits (Rashid et al., 2007).

The technique thus aids the identification of potential parents for hybridization based on genetic information and knowledge of their combining ability.

Different workers have carried out combining ability studies in okra with different outcomes. Shusmita and Das (2003) reported that additive gene action was more important in the inheritance of inter-node length, number of nodes, fruit length, fruit diameter, plant height and pod yield. Kumar (2006), however, noted that non-additive gene action was largely responsible for the expression of plant height, number of branches, pod weight, pod length and pod yield. Rewale et al. (2003) in their study identified three lines; DVR-4, SOH-02 and Arka Anamika that had favourable GCA effects for yield and related traits. Prakash et al., (2002) in a line × tester analysis involving 7 lines and 3 testers reported the preponderance of non-additive gene action over additive gene action for all the measured traits. They further identified 4 out of 10 parents with good GCA for pod yield while 2 of the 21 hybrids exhibited favourable SCA effects for the majority of the characters.

The objectives of this study were to determine the gene action controlling the inheritance of some important agronomic traits in okra and to determine the combining ability of the parents used in the study.

Contact address: Muyideen Oluseyi Olayiwola, Olabisi Onabanjo University, College of Agricultural Sciences, Department of Crop Production, Ago-iwoye, Nigeria, Phone: +2348033990585, e-mail: ollyrichie@gmail.com; muyideen.olayiwola@oouagoiwoye.edu.ng;

Materials and methods

Planting materials and generation of hybrids

The study was carried out at the Teaching and Research Farm of the Federal University of Agriculture, Abeokuta, Nigeria (latitude 7° 29′ N and longitude 3° 3′ E). Seven inbred lines sourced from the gene bank of the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, were utilised for this study. Five were designated as lines and two as testers based on their previous performance (Oyetunde, 2015). Each male (Tester) parent was crossed to each female (Line) parent to generate ten crosses. Each genotype was sown in single row plot of 4 m length with 0.6 m as inter-row and 0.4 m as intra-row. Planting was done in succession to ensure synchronization of flowering, and crosses were made accordingly. The parents were selfed to ensure availability of fresh seeds for evaluation. Harvesting was done after the pods had dried on the mother plants to ensure viability of seeds for evaluation.

Field evaluation and data collection

The ten hybrids and seven parents were sown at the rate of two seeds per hole and later thinned to one plant per stand. The experiment was laid out in a randomized complete block design (RCBD) with three replications separated by 1 m to enhance easy movement during field operations. Each plot was a 4.5 m long single row with 0.70 m as inter-row and 0.45 m as intra-row. Three and six weeks after planting, NPK 15 : 15 : 15 was applied at a recommended rate of 60 kgN. ha^{-1} using the side placement method. Weeding was done manually as necessary and insect pests were controlled using cypermethrin at the rate of 4 ml.$litre^{-1}$ of water.

Observations were made on number of days to 50% flowering, plant height (cm), stem diameter (cm), number of branches, number of pods, pod weight (g), pod length (cm), pod width (cm) and pod yield (kg) from representative plants on each plot.

Data analysis

Data collected were subjected to line × tester analysis as described by Singh and Chaudhary (1985) to determine the gene action underlying the inheritance of the measured characters. The GCA and SCA and their corresponding standard errors were then computed for all traits. The two genetic parameters were tested for significance using their standard errors as outlined by Singh and Chaudhary (1985).

Table 1 Mean squares from ANOVA of characters evaluated in okra using line by tester analysis

Source	Df	50%F[a]	NoB	StG	PtH	PdL	PdWgt	NPdPt	YPt
Replicate	2	31.82	7.19	0.59**	1652.93*	0.13	0.03	291.25**	59627.83**
Genotype	16	126.81**	3.44	0.1	1955.48**	0.94**	2.29	68.59**	6840.68
Parent (P)	6	122.65**	2.82	0.05	1562.86**	1.54	3.68	92.95**	7277.42**
Cross (C)	9	88.46**	3.1	0.04	937.29**	0.61	1.62	56.56**	4765.63**
PvsC	1	496.98**	10.18	0.94	13474.88**	0.21	0.02	30.74	22895.76**
Line (L)	4	81.45**	1.1	0.02	813.69**	0.9	2.38	32.06	1849.19**
Tester (T)	1	202.80**	7.7	0.04	329.35**	1.01	1.88	339.36**	29371.92**
L × T	4	66.88*	3.95	0.06	1212.88**	0.23	0.79	10.35	1530.49**
Error	32	17.99	2.7	0.05	626.82	0.17	2.24	23.37	4089.23

Table 2 General combining ability (GCA) of the parental lines evaluated for 8 agronomic traits in okra

Line	50%F[a]	NoB	StG	PtH	PdL	PdWdt	NPdPt	YPt
NGB00303	4.13*	0.49	0.03	12.64	-0.50**	0.03	-0.76	-4.36
NGB00346	2.8	-0.53	0.08	5.27	-0.28*	0	-1.58	-10.92
NGB00347	0.3	-0.03	-0.08	-13.25	0.35*	-0.07	-2.53	-24.86
NGB00297	-2.37	-0.29	-0.01	6.85	0.05	0.08*	2.34	36.83
NGB00356	-4.87**	0.36	-0.02	-11.51	0.38*	-0.04	2.54	3.32
SE line	1.73	0.67	0.09	10.22	0.17	0.05	1.97	34.90
Tester								
NGB00326	-2.60*	0.51	0.04	-3.31	0.18*	-0.02	3.36**	41.06
NGB00302	2.60*	-0.51	-0.04	3.31	-0.18*	0.02	-3.36**	-41.06
SE tester	1.1	0.42	0.06	6.46	0.11	0.03	1.25	21.66

(for tables 1 and 2) *,** significant at 5 and 1% probability levels, respectively
50%Fa – days to 50% flowering, NoB – number of branches, StG – stem diameter (cm), PtH – plant height (cm), PdL – pod length (cm), PdWdt – pod width (cm), PdWgt – pod weight (g), NPdPt – number of pods, YPt – pod yield ($kg{\cdot}ha^{-1}$)

Table 3 Specific combining ability (SCA) effects of 10 hybrids evaluated for 8 agronomic traits in okra

Cross	50%F[a]	NoB	StG	PtH	PdL	PdWdt	NPdPt	YPt
NGB00303 × NGB00326	0.93	0.41	0.1	19.16	-0.2	0.02	-0.4	24.60
NGB00303 × NGB00302	-0.93	-0.41	-0.1	-19.16	0.2	-0.02	0.4	-24.60
NGB00346 × NGB00326	1.93	0.83	-0.07	-6	-0.18	0.02	0.15	-7.56
NGB00346 × NGB00302	-1.93	-0.83	0.07	6	0.18	-0.02	-0.15	7.56
NGB00347 × NGB00326	-4.23*	-1.01	-0.04	0.21	0.25	-0.08	0.34	-12.88
NGB00347 × NGB00302	4.23*	1.01	0.04	-0.21	-0.25	0.08	-0.34	12.88
NGB00297 × NGB00326	-2.57	0.49	-0.09	-19.25	0.12	0.01	1.77	19.37
NGB00297 × NGB00302	2.57	-0.49	0.09	19.25	-0.12	-0.01	-1.77	-19.37
NGB00356 × NGB00326	3.93	-0.72	0.11	5.88	0.02	0.02	-1.86	-23.55
NGB00356 × NGB00302	-3.93	0.72	-0.11	-5.88	-0.02	-0.02	1.86	23.55
SE (L×T)	2.45	0.95	0.13	14.45	0.24	0.07	2.79	48.46

*,** significant at 5 and 1% probability levels, respectively.
50%F[a] – days to 50% flowering, NoB – number of branches, StG – stem diameter (cm), PtH – plant height (cm), PdL – pod length (cm), PdWdt – pod width (cm), PdWgt – pod weight (g), NPdPt – number of pods, YPt – pod yield (kg·ha^{-1})

Table 4 Mean performance of the 17 okra genotypes evaluated for eight agronomic traits

Genotype	50%F[a]	NoB	StG	PtH	PdL	PdWdt	PdWgt	NPdPt	Ypt
NGB00326 (T1)	68.33	6.87	1.46	143.43	5.30	1.93	8.77	20.23	803.45
NGB00302 (T2)	70.00	5.73	1.45	136.87	4.33	2.37	11.17	10.10	504.45
NGB00303 (L1)	56.33	5.90	1.22	129.07	5.93	2.13	12.07	25.47	1115.69
NGB00346 (L2)	55.67	6.87	1.16	103.30	5.47	2.27	11.10	21.13	889.44
NGB00347 (L3)	56.00	7.90	1.37	146.37	6.47	1.90	10.60	25.63	847.61
NGB00297 (L4)	55.67	5.40	1.38	87.37	5.50	2.13	9.77	26.10	1100.94
NGB00356 (L5)	57.00	7.67	1.49	106.10	6.30	2.00	11.40	21.97	896.53
NGB00303 × NGB00326 (L1×T1)	68.67	8.93	1.81	183.30	4.97	2.20	9.07	25.30	1253.73
NGB00346 × NGB00326 (L2×T1)	68.33	8.33	1.68	150.77	5.20	2.17	10.20	25.03	1130.82
NGB00347 × NGB00326 (L3 × T1)	59.67	7.00	1.56	138.47	6.27	2.00	10.50	24.27	1069.69
NGB00297 × NGB00326 (L4 × T1)	58.67	8.23	1.57	139.10	5.83	2.23	11.50	30.57	1367.89
NGB00356 × NGB00326 (L5 ×T1)	62.67	7.67	1.76	145.87	6.07	2.13	11.17	27.13	1125.27
NGB00303 × NGB00302 (L1 × T2)	72.00	7.10	1.53	151.60	5.00	2.20	10.73	19.37	836.78
NGB00346 × NGB00302 (L2 × T2)	69.67	5.67	1.76	169.40	5.20	2.17	10.90	18.00	918.03
NGB00347 × NGB00302 (L3 × T2)	73.33	8.00	1.56	144.67	5.40	2.20	10.67	16.87	890.69
NGB00297 × NGB00302 (L4 × T2)	69.00	6.23	1.68	184.23	5.23	2.27	11.67	20.30	984.15
NGB00356 ×NGB00302 (L5 × T2)	60.00	8.10	1.47	140.73	5.67	2.13	10.97	24.13	1014.03
Mean	63.59	7.15	1.52	141.21	5.54	2.14	10.72	22.45	985.25
SE	1.58	0.26	0.04	6.19	0.14	0.03	0.21	1.16	48.16

50%Fa – days to 50% flowering, NoB – number of branches, StG – stem diameter (cm), PtH – plant height (cm), PdL – pod length (cm), PdWdt – pod width (cm), PdWgt – pod weight (g), NPdPt – number of pods, YPt – pod yield (kg·ha^{-1})

Results and Discussion

A detailed understanding of the inheritance of important agronomic traits in okra is central to the improvement of the vegetable crop. Identification of ideal parents, development and promotion of hybrids would not only increase yields, but also serve as sustainable sources of useful genetic variability for continuous improvement program. Hybridization between genotypes that are not genetically diverse or with little genetic variation might not give higher heterotic value in F_1 and may result in narrow range of variability in the segregating F_2 population. The significant differences revealed among the genotypes for

days to flowering, plant height, pod length and number of pods (Table 1) underscored the differential performance among the parents and their hybrids which is an indication of substantial genetic variability that could be explored for future improvement (Weeraskar, 2006).

A significant line or tester and line × tester effects on a trait is an indication of the gene action conditioning the trait under consideration and the worth of the parents and hybrids for that trait. For instance, a significant line or tester effect implies that such trait is under additive gene control while a significant line × tester effect suggests that the character is under non-additive gene control. When both are significant, that is, line/tester and line × tester, the effect with the highest mean square would be considered more important in the inheritance of the concerned trait. In our study, line or tester and line × tester effects were significant for days to 50% flowering, plant height and pod yield which implies that breeding strategies that could take advantage of both additive and non-additive gene effects should be considered in the improvement of the traits listed. However, based on the components of mean squares, additive gene action is more important than non additive in the control of earliness and pod yield implying that faster progress may be obtained through recurrent selection scheme. Plant height would be best improved through heterotic breeding as suggested by the higher importance of non-additive gene action in its inheritance.

Secondly, the significant tester effect for some of the evaluated characters confirmed the contrasting nature of NGB00326 and NGB00302 that were used as testers in this study. This implies that the testers have the potential to discriminate among the lines in terms of the values for the measured traits. That the line had significant effect on some traits indicated that the lines showed varied performance (GCA) in hybrid combinations with the testers while the significant line × tester effect points to differential performance of specific crosses. Furthermore, the parents and crosses that showed significant combining ability effects in the preferred direction are high combiners and valuable sources of favourable alleles for the improvement of the character involved (Reddy et al., 2013; Badu-Apraku et al., 2016). For instance, the negative significant GCA effect associated with NGB00356 and NGB00326 (Table 2) for days to 50% flowering shows the worth of this line in the development of early maturing okra genotypes. Tester NGB00326 had a positive and significant GCA effect for number of pods indicating its value as a candidate parent in the development of high yielding okra genotypes (Prakash et al., 2002; Rewale et al., 2003).

All the hybrids showed non-significant SCA effects (Table 3) for all characters except days to 50% flowering in hybrids NGB00347 × NGB00326 (earliness) and NGB00347 × NGB00302 (late maturity). This may be due to the lack of complementation of the parental genes and/or because okra is a self-pollinated crop, leading to little heterotic advantage. Reddy et al., (2013) had earlier reported that self-pollinated crops such as okra exhibit low hybrid vigour. However, NGB00297 × NGB00326 had the highest positive SCA effect for pod yield and also the highest pod yield (Table 4). Thus, NGB00356 and NGB00326 could be considered as sources of alleles for the development of early maturing okra types while the cross NGB00297 × NGB00326 could be exploited for production of high yielding okra genotypes.

Conclusion

The study revealed differences among the parental lines for some of the characters studied and confirmed the contrasting features of the testers. Furthermore, the inheritance patterns of the various agronomic traits were determined and the potentials of the parental lines as sources of desirable alleles for okra improvement were documented.

References

AHIAKPA, J.K. – KALEDZI, P.D. – ADI, E.B. – PEPRAH, S. – DAPAAH, H.K. 2013. Genetic diversity, correlation and path analyses of okra (*Abelmoschus* spp. (L.) Moench) germplasm collected in Ghana. In International Journal of Development and Sustainability, vol. 2, 2013, no. 2, pp. 1396–1415.

ARORA, D. – JINDAL, S.K. – GHARI, T.R. 2007. Generation mean analysis for earliness related traits in Okra (*Abelmoschus esculentus* (L). Moench). In Journal Genetics and Breeding, vol. 61, 2007, pp. 1–7.

BADU-APRAKU, B. et al. 2015. Heterotic responses among crosses of IITA and CIMMYT early white maize inbred lines under multiple stress environments. In Euphytica, vol. 204, 2015, no. 3, pp. 245–262. doi: 10.1007/s10681-015-1506-0.

KEMPTHORNE, O. 1957. An introduction to genetic Statistics. New York : John Wiley and Sons, 1957.

KUMAR, N. 2006. Breeding of Horticultural Crops. New Delhi : New India Publishing, 2006.

KUMAR, S. – DAGNOKO, S. – HAOUGUI, A. – RATNADASS, A. – PASTERNAK, D. – KOUAME, C. 2010. Okra (*Abelmoschus* spp) in West and Central Africa: Potential and progress on its improvement. In African Journal of Agricultural Research, vol. 5, 2010, no. 25, pp. 3590–3598.

NWANGBURUKA, C. C. – DENTON, O.A. – KEHINDE, O.B. – OJO, D.K. – POPOOLA, A.R. 2012. Genetic variability and heritability in cultivated okra [*Abelmoschus esculentus* (L.) Moench]. In Spanish Journal of Agricultural Research, vol. 10, 2012, no. 1, pp. 123–129.

OYETUDE, O.A. 2015. Genetics and Inter-character Relationships in a Population of Sixteen F_1-hybrids of Okra (*Abelmoschus esculentus* L. (moench)), arising from Biparental crosses. M. Agric. Abeokuta : Thesis, Federal University of Agriculture, 2015.

PRAKASH, M. – KUMAR, M.S. – SARAVANAN, K. – KANNAN, K. – GANESAN, J. 2002. Line × tester analysis in okra. In Annals of Agricultural Research 23: 233-237.

RASHID, M. – CHEEMA, A. A. – ASHRAF, M. 2007. Line × tester analysis in basmati rice. In Pakistan Journal of Botany, vol. 39, pp. 2035-2042.

REDDY, M. T. – BABU, K.H. – GANNESH, M. – BEGUN, H. – DILIPHABU, J. – REDDY, R. S. K. 2013. Gene action and combining ability of yield and its components for late *kharif* season in okra (*Abelmoschus esculentus* (L.) Moench). In Chilean Journal of Agricultural Research, vol. 73, 2013, no. 1, pp. 0718–5839.

REWALE, V. S. – BENDALE, V.W. – BHAVE, S. G. – MADAV, R. R. – JADHAV, B. B. 2003. Combining ability of yield and yield components in okra. In Journal of Maharashtra Agricultural University, vol. 28, 2003, pp. 244–246.

SHUSMITA, M. – DAS, N.D. 2003. Combining ability studies in okra. In Journal of Interacademicia, vol. 7, 2003, no. 4, pp. 382–387.

SINGH, R.H. – CHOUDHARY, B.D. 1985. Biometrical methods in quantitative genetics analysis. New Delhi : Kalyani Publishing, 1985.

WEERASKAR, D. 2006. Genetic analysis of yield and quality parameters in okra (*Abelmoschus esculentus* (L) Moench). Dharward : M. Agric Thesis, University of Agricultural Science, 2006.