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Articles and Statements

A Study of the Physiological Properties of Some Phytopathogenic Bacteria

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Abstract

The physiological features of nutrition of some phytopathogenic bacteria were studied. Attention was focused on the selection of nutrient media, for virulence is in correlation with the nutrition of bacteria. Natural and synthetic media were selected to obtain a large quantity of biomass, accelerate growth, activate some enzymic systems and pigmentation, as well as to ensure long-term preservation of cultures in the condition of intensive vital activity. In connection with the dynamics of the development of the bacterial infection in plants the duration of phase development in different media was studied.

Keywords: phytopathogenic bacteria, nutrient media, virulence, enzymic system, strains, inoculums, stationary phase, biomass.

1. Введение

Связь между питанием фитопатогенных бактерий и вирулентностью отмечают многие авторы. Эти бактерии могут использовать, с одной стороны, химические соединения, входящие в состав растения и, с другой стороны, промежуточные и конечные продукты метаболизма растения. При изучении проникновения микробной инфекции и заболеваний в растение важную роль играет установление особенности питания фитопатогенных бактерий. С помощью этого возможно выявить «агрессивные» механизмы, способствующие заболеванию растения и последующему развитию, такие как ферменты, токсины, продукты метаболизма (Дьяков, 2012 Шкаликов и др., 2005).

Изучение питательных особенностей бактериальных заболеваний и закономерностей роста и развития культур является важным вопросом при определении взаимоотношений между растением-хозяином и паразитом-бактерией.

Целью нашей работы является изучение физиологических свойств некоторых фитопатогенных бактерий, распространённых в Грузии, и установление их питательных особенностей. Т.к. в процессе развития микробной инфекции учувствуют разные химические соединения и продукты метаболизма, был интересен выбор питательных сред растения на основании главных химических соединений и продуктов метаболизма, которые, с одной стороны ставят барьер, а, с другой стороны предоставляют пищу для

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фитопатогенных бактерий. С помощью использования питательных сред разного содержания можно изучить закономерность роста и развития фитопатогенных бактерий, выявить их потенциальные возможности с точки зрения питания, темпа и интенсивности развития, появления ферментов и протекания других процессов.

2. Материалы и методы

Объектом для исследования были выбраны местные штаммы фитопатогенных бактерий, полученных из микробиологической лаборатории института защиты растений имени Л. Канчавели. Использование указанных бактерий для опыта было обусловлено тем, что они часто встречаются в условиях умеренного климата Грузии и вызывают заболевания цитрусов, чая, фруктов и ягод, овощей и других культур.

В процессе работ были использованы следующие пищевые среды и методы: мясопептонный агар (МПА), мясопептонный бульон (МПБ), мясопептонный желатин (МПЖ), аграризированные среды, изготовленные из разных растений (картофель, морковь, капуста, помидоры), водный агар, коническая среда, среда Ушинского, фермерская среда, среда Омелянского, синтетическая нитратная среда, среда Георгия и Поэ, молочный агар, агаризированное пивное сусло, крахмальная среда. МПА, МПБ, МПЖ, молочный агар использовался, как источник энергии, C и N для изучения интенсивности роста и динамики культур, получения избыточной биомассы, активизации протеолизных ферментов, изучения пигментации, растительный агар, среда Омелянского, крахмальная среда, пивной агар для изучения ферментации углевода и для активизации соответствующих ферментов, для изучения роста и развития, динамики и интенсивности культур.

Коническую среду, среду Ушинского, среду Георгия и Поэ, синтетическую нитратную среду для установления закономерности в основном использовали источником питания аминокислот, органических кислот и разных неорганических солей.

pH пищевой среды определяли, используя индикаторную бумагу, рассеивая количество живых микробов на прочной пищевой среде, подсчитывая общее количество микробов в камере Горяева, биомассу пробных бактерий – нефелометрированием, количество инокулата 2 % пищевой среды, титр – $5x10^8$ клеток/мл, возраст – 5 дней, температура культивации – 20°C (Методы определения болезней..., 1987; Sinclair, Dhingra, 1995; Ковальчук и др., 2010; Тихонов и др., 2005).

3. Результаты и их обсуждение

Показатели интенсивности роста пробных бактерий в разной пищевой среде согласно оптической плотности указаны в таблице. Показатель оптической плотности во всех вариантах взят в стационарной фазе развития культуры, т.к. мы интересовались максимальным количеством биомассы.

Таблица 1. Биомасса некоторых фитопатогенных бактерий в разных пищевых средах, согласно оптической плотности

№	Использованная пища	Pseudomonas tumefaciens	Pectobacterium phytophthorum	Pectobacterium aroideae	Pectobacterium carotovorum	Xanthomonas vesicatoria	Xanthomonas campestris
1	МПА	0,9	1,3	1,3	1,2	1,0	0,7
2	МПБ	0,7	1,0	1,2	1,2	1,0	0,8
3	Водный агар	0,1	0,3	0,3	0,3	0,2	0,1
4	Коническая среда	0,4	0,7	0,5	0,7	0,5	0,3
5	Среда Ушинского	0,8	1,2	0,9	0,9	0,7	0,6
6	Фермерская среда	0,4	0,5	0,5	0,5	0,4	0,3
7	Среда Омелянского	0,35	0,9	1,0	1,0	0,8	0,8

8	Синтетическая нитратная среда	0,4	0,6	0,6	0,6	0,4	0,3
9	Среда Георгия и Поэ	0,6	0,7	0,8	0,8	0,6	0,5
10	Молочный агар	5,0	5,5	5,7	5,8	0	4,8
11	Аграризованное пивное сусло	4,2	4,4	4,5	4,4	4,0	3,9
12	Картофельный агар	1,9	2,3	2,2	2,4	1,8	1,7
13	Капустный агар	0,7	1,0	0,9	0,9	1,1	1,2
14	Морковный агар	0,7	0,9	0,8	0,8	1,0	1,0
15	Томатный агар	0,7	0,8	0,9	0,9	0,8	0,7

Из таблицы 1 следует, что самой лучшей средой для роста и развития бактерий является молочный агар. В случае его использования количество бимассы в 2-3 раза больше показателей полученных в других более распространенных пищевых средах (МПА, картофельный агар). Молочный агар был впервые нами использован для культивирования фитопатогенных бактерий. Это лучшая среда, как для получения большого количества бактериальной биомассы, так и для длительного хранения в жизнеспособном состоянии. Кроме этого, в указанной среде прекрасно проявляются пигменты, что очень важно в изучении роли пигментов при токсическом действии бактерий. При культивации бактерий на молочном агаре (даже 1 пассаж) значительно активировались протеолизные ферменты, а в результате выросших в таких условиях культур растёт качество и скорость сжижения МПЖ. Значительное количество биомассы было получено в результате использования пивного агара в качестве пищи, но по интенсивности роста оно меньше, чем на молочном агаре. Растительный агар также не даёт интенсивного развития культур, как и синтетические сферы. Рост в случае разных культур неравномерный. Например, на сфере Ушинского рост *Pectobacterium phytophthorum* был более сильный. Глюкоза подавляла развитие *Pseudomonas tumefaciens*, а *Xanthomonas campestris* почти во всех случаях отставала интенсивностью роста от других культур.

При изучении бактериальных заболеваний большое значение имеет анализ динамики роста и развития культур, так как этапы развития инфекционного процесса связаны с закономерностью роста и развития (Тихонов и др., 2005). В связи с этим были изучены фазы роста и развития пробных бактериальных культур в разных условиях культивирования. Изучение производили на богатых пищевых сферах (МПА, МПБ, картофельный агар, пивной агар, молочный агар). Самым долговременным считался лаг-период при использовании МПА и картофельного агара, его продолжительность составляет 4 дня. А продолжительность лаг-фазы на молочном агаре и пивном агаре уменьшена не превышает 16-18 часов, но аналогичные условия развития растительных бактерий даёт твёрдая пища, поэтому очень важны результаты, полученные в этих условиях.

Важным фактором является также продолжительность и интенсивность стационарной фазы. В случае указанных культур на молочном агаре стационарная фаза продолжается до 1 месяца и бактериальный рост долгосрочно остаётся в жизнеспособном состоянии. Также долго продолжается стационарная фаза при культивировании на пивном агаре. На МПА и картофельном агаре стационарная фаза достигает 8-12 дней. При использовании МПБ рост бактериальных культур уменьшается, так же уменьшается и период их жизнедеятельности.

Для культивирования фитопатогенных бактерий использовали до 20-ти наименований универсальных и синтетических питательных сред. Особенно интенсивный рост был получен на молочном агаре и пивном агаре. Указанные среды мы рекомендуем для получения избыточного количества биомассы культур фитопатогенных бактерий, для усиления роста, а также для длительного хранения бактериальных культур в жизнеспособном состоянии, для пигментации и активации соответствующих ферментных комплексов. Установлена динамика роста и развития бактерий опытного штамма, особенно продолжительность фаз развития на разных питательных средах.

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Изучение физиологических свойств некоторых фитопатогенных бактерий

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Аннотация. Изучены физиологические особенности питания некоторых фитопатогенных бактерий. Основное внимание уделено подбору питательных сред, так как вирулентность находится в коррелятивной связи с питанием бактерий. Подобраны естественные и синтетические среды для получения большого количества биомассы, ускорения роста, активирования пигментации и некоторых ферментных систем, а также длительного сохранения культур в состоянии активной жизнедеятельности. В связи с динамикой развития бактериальной инфекции в растениях изучена длительность фаз развития культур на разных средах.

Ключевые слова: фитопатогенные бактерий, питательная среда, вирулентность, ферментные системы, штаммы, инокулат, стационарная фаза, биомасса.

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Bulbs Surface-Sterilization Protocol for *Tulipa julia* C. Koch from Turkey

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Abstract

Tulipa julia is an ornamental important plant and a wide geographical distribution eastern of Turkey. However, due to habitat loss and illegal over collection in the wild it is included as a vulnerable species. The development of a protocol for *Tulipa julia* bulblet propagation *in vitro* may be useful for reintroducing plants in their natural habitats, and for germplasm conservation. A difficult problem encountered during the establishment of an *in vitro* culture is explants disinfection, especially when working with endangered species, from which explant availability is restricted. Thus, the establishment of a sterilization protocol is crucial for the initiation and success of bulblets micropropagation system for *Tulipa julia*. This study was to evaluate the effect of sodium hypochlorite concentrations and treatments time in bulbs surface disinfection, tissue sensitivity and development. Sodium hypochlorite solutions (2 or 3 %, 20 or 25 min; 4 or 5 %, 30 or 35 min) were effective in eliminating bulbs superficial contaminants. There was significant difference among the effective sterilization sodium hypochlorite concentrations and treatments time in relation to surface sterilization bulbs of *Tulipa julia*. Also, no damage to bulbs tissues were observed. Surface sterilization of bulbs, for initiation of an *in vitro* culture, required higher concentrations of sodium hypochlorite (4 or 5 % NaCl, 30 or 35 min) for controlling fungal and yeast contamination, compared to bulbs sterilization.

Keywords: *Tulipa julia*, surface sterilization, *in vitro* culture.

1. Introduction

Turkey is one of the richest countries in variability of flora, it has more than 10000 plant taxa about 3000 of which are endemic. 'There are about 600 species of flower bulbs in Anatolia' (Arslan et al., 2002) and many of them are known as ornamental and medicinal plants (Atay, 1996). 'A number of these geophytic taxa have been exported from Turkey for a long time' (Arslan et al., 2002). Tulip (*Tulipa* L.) genus is a Monocotyledona and belongs to the Liliaceae Juss. Family and comprises more than 100 species in the world (Hall, 1940). In Turkey, *Tulipa* was divided into two subgenera and they represented in total 19 taxa (Eker et al., 2014). *Tulipa* L. taxa are among significant plants widely used as ornamentals, they have been originated in Eastern countries and Iran and Turkey were introduced in Europe (Matin, 1998). Tulips are unique representative of plants; their significance has always been exceptional. Tulips are important bedding bulbous ornamental plants that widely used in the park and gardens and widely cultivated in world and in Turkey for cut flower, potted plant, landscaping. The Tien Shan and Pamir-Alay mountain ranges in central Asia are considered the primary gene centers for *Tulipa* species' (Botschantzeva, 1962),

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with the Caucasus as a secondary center. They are popular spring-flowering garden plants; millions of bulbs are sold annually and over 5.000 cultivars are registered (Van Scheepen, 1996).

Several species are in cultivation, but they cover less than 7 % of the total tulip area in the Netherlands. The boundaries between taxa of various ranks are still a subject of dispute (Zonneveld, 2009). The vegetative propagation of *Tulipa* taxa are effective, but creation of new tulip cultivars is especially time-consuming because tulip seedlings begin to blossom only after seven years. Researchs were carried out to make this period shorter, but no positive results were received. The process of creating a new cultivar takes long years because not only the period from sowing till blossom of seedlings is long, but also a long period is needed for bulb propagation till standard extents of industrial production' (Baliūnienė, Juodkaitė, 1991). 'It is commercially propagated through asexual reproduction by using bulbs, but the effciency of this process is low' (Lenard, De Hertogh, 1993).

In vitro storage organ formation (tuberization) has been broadly studied with most of geophytes (Podwyszyn´ska, 2012), like *Scilla siberica* subsp. *armena* (Ozdemir et al., 2016). Most geophytes require certain induction factors for storage organ formation, e.g. *Tulipa* taxa require low temperatures, some onion genotypes require long photoperiods, and potatoes need short photoperiods and low night temperatures. 'However, numerous reports suggest that two factors induce an in vitro storage organ formation in most geophytes, including bulbous plants: a high sucrose concentration in in vitro media and a sharp reduction in endogenous gibberellin levels in response to environmental cues' (Podwyszyn´ska, 2012). The latter has been approved by demonstrations that application of gibberellin biosynthesis inhibitors stimulates bulb formation in garlic (Kim et al., 2003), lily (Kumar et al., 2005) and tulip (Podwyszyn´ska, 2006). Several other plant growth regulators also have extensivel reported stimulatory effects on bulbing, including auxins (Van Aartrijk, Blom-Barnhoorn, 1981), ethylene (Taeb, Alderson, 1990) and jasmonates (Podwysyn´ska, 2006). However, there are conflicting indications of the roles of cytokinins in bulb formation *in vitro*. Benzyladenine stimulated the bulb development in *Lilium longiflorum* (Easter lily, Bermuda lily, trumpet lily) and *Urginea maritima* L. (Baker) (Nhut, 1998) but inhibited bulb formation in *Narcissus jonquilla* L. (Chow et al., 1992). A high cytokinin to auxin ration improved the bulb production in *Hyacinthus* L. (Liliaceae) taxa (Kim et al., 1981) and *Fritillaria fleischeriana* Steudel et Hochst. ex Schultes et Schultes Fil. (Mirici et al., 2005). On the other hand, low exogenous cytokinin to auxin ratio reportedly promote bulb growth of *Hippeastrum* (Amaryllidaceae) taxa (Huang et al., 2005)

Investgation using *in vitro* methods to examine efficient multiplication rates in the *Tulipa* taxa has been in progress for many years. 'Unfortunately, the laboratory techniques employed in its propagation continue to produce low yields' (Maglanka, Bach, 2010). Organogenesis is a type of plant regeneration that can be used in clonal propagation. In bulbous plants like tulips this micropropagation method results in the formation of adventitious shoots or bulbs (Ghaffor et al., 2004). 'The organs are formed directly on explants or indirectly via callus tissue' (Liu, Yang, 2012). Among geophytes, organogenesis can occur on various explants, including on buds, pedicels, seeds (Ghaffor et al., 2004). 'In vitro cultures of the tulip have been initiated mainly from chilled bulbs; though some experiments have used non-chilled plant material' (Ptak, Bach, 2007).

2. Relevance

The present investigation was undertaken to ensure that large numbers of clean explants should survive sterilization. In the present study two concentration of sodium hypochlorite % 2-3 and % 4-5 (NaCl) were used.

3. Material and methods

The bulbs of *Tulipa julia* were collected natural habitats, during within field work project (BAP –TBMYO.2016.00.001) from Bingol-Solhan province by taxonomist O. Kılıç. All of using this study bulbs washed under running tap water for 30 min. before from the study. Different concentrations of sodium hypochlorite (2 %, 3 %, 4 %, 5 %) were used for 20, 25, 30, 35 min. inside in the laminar flow air cabine, and a final wash with autoclaved distilled water 5 times. All *Tulipa julia* bulbs cultivated in MS (Murashige, Skoog, 1962) medium. Cultures were incubated in the

controlled conditions of temperature (24±1 °C) and light intensity (2000-2500 lux for 16 h); the experiments were replicates 3 times.

4. Discussion

It is always a big challenge to avoid contamination and establishment of aseptic cultures from the field grown plants which are always at high risk of internal and external contamination. The present investigation was carried out to optimize sterilization protocol for fast multiplication of *Tulipa julia*. There was significant difference among the effective sterilization sodium hypochlorite concentrations and treatments time in relation to surface sterilization bulbs of *Tulipa julia*. At lower concentrations and little treatments time (2 or 3 %, 20 or 25 min.) of sodium hypochlorite when used showed less in sterilizing the *Tulipa julia* bulbs but higher concentrations and high treatments time (4 or 5 % NaCl, 30 or 35 min) of sodium hypochlorite when used showed effective in the sterilizing of bulbs. Also, no damage to bulbs tissues were observed. Surface sterilization of bulbs, for initiation of an *in vitro* culture, required higher concentrations and treatments time of sodium hypochlorite (4 or 5 % NaOCl, 30 or 35 min) for controlling fungal and yeast contamination, compared to bulbs sterilization.

Comparing salt types, NaCl proved to be superior compared to other salts for bulbous growth as used in this study. 'NaCl is normally expected to have an adverse effect on plant growth and development due to suppressed cell division and restricted growth activities' (Bohnert, Jensen, 1996). Accumulation of Na+ and Cl– in tissues led to toxicity (Karimi et al., 2009) in the cells' cytoplasm, which affected distinct biochemical and physiological processes (Jampeetong, Brix, 2009). Our results revealed that bulblets tolerated a concentration of 4 % or 5 % NaOCl, 30 or 35 min, which ultimately promoted the bulbous growth more efficiently. The positive response of bulblets to a specific salt concentration might be due to higher tolerance showed by plants at maturity, or might depend on the type of organ (Jenks et al., 2007) used in the study. Similarly, negative effects of a higher KCl concentration lead to salt stress and may affect plant growth and development by causing callus induction, necrosis, and shoot regeneration, in line with the findings of Zahid et al. (2014). The results clearly show that the addition of salts at low concentrations for a specific time can be used to increase bulblet size and to harden the bulblets. 'Acclimatization of in vitro regenerated bulblets is the most challenging task due to smaller size and dormancy found in the in vitro regenerated bulblets' (Petric et al., 2011). Therefore, optimum bulblet size with adequate rooting is a prerequisite for successful acclimatization. Researchers adopted different approaches in order to increase bulblet size prior to acclimatization.

Losses due to contamination under *in vitro* conditions average between 3 and 15 % at every subculture in the majority of commercial and scientific plant tissue culture laboratories, the majority of which is caused by fungal and bacterial contaminant (Leifert et al., 1989). Therefore, to ensure the reduction of the contaminants as well as high survival rate of explants, it requires efficient aseptic techniques in tandem with effective sterilization methods before subjecting them for tissue culture study (Srivastava et al., 2010). Sodium hypochlorite is a very effective sterilant and extensively used to stimulate reduce contamination in cultures (Nongalleima et al., 2014).

5. Conclusion

In the present investigation NaCl (4 or 5 %) for 30 or 35 min was found more effective for sterilization and further *in vitro* response of bulbs. The previous research also suggests that NaCl is an effective sterilizing agent (Chengalrayan et al., 2005). Our finding suggests the use of sodium hypochlorite for higher time period to obtain aseptic culture of *Tulipa julia* bulbs. The findings will provide a good base for effective and quick sterilization of *Tulipa julia* bulbs especially when they are procured from field grown plants.

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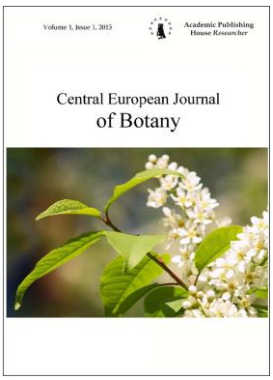

Flowering Phenology of Some Plants Grown in Zeve Campus, University of Yüzüncü Yıl (Van-Turkey)

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Abstract

The science searching the developmental process like the budding, leafing and flowering timing and period of plant is called phenology. In other terms, phenology is broadly defined as the study of recurring plant and animal life cycle stages, especially their timing and relationships with weather and climate. Phenology (from the Greek phainein, to show or appear) is the study of the timing of these life-history events. In plants, bud-burst, leaf-expansion, abscission, flowering, fertilisation, seedset, fruiting, seed dispersal and germination all take place in due season. In the present study is aimed to investigate the flowering phenology of some plants grown in Van located in the surrounding of the world's biggest soda lake where terrestrial climate, sunny days of the year and steppe vegetation are dominant. Investigation of flowering phenology is important in terms of developing strategies against freezing especially for cultivated plants. The factors effecting flowering phenology are; temperature, photoperiod, altitude, location and fertilizing of plants. In this study, flowering time of some plants were determined in 2013, 2014 from Zeve Campus area of Yüzüncü Yıl University located in B9 square where the altitude is between 1650-1680 m. In this study flowering period of 132 plant species belong to 33 families were determined.

Keywords: plant, flowering time, phenology, Van.

1. Introduction

'Phenology is the study of the timing of recurring biological phases, the causes of their timing with regard to biotic and abiotic forces, and the interrelation among phases of the same or different species' (Lieth, 1974). Early forecasting of the phenological phases of wild and cultivated plant taxa are of great support to various sectors of human activity, particularly for all agricultural practices. Phenology science plays an important role in many processes that are relevant for agriculture, horticulture and silviculture: suitability for production and yield potential, frost damage prevention, length of growing season and frost free days, epidemiology of diseases and pests, timing of sowing, sprinkling, harvesting, isectiside and herbicide use, irrigation and many other areas. 'Predicting the onset of pollen season is of particular importance to people allergic to given pollen, who can on the base of forecast start anti-allergic treatment several days before pollination, and thereby optimizing its effectiveness' (Rodriguez-Rajo et al., 2003).

'Distinct changes in air temperatures since the end of the 1980s have led to clear response in plant phenology in many parts of the world. Since then many phenological papers report on

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trends in the timing of spring events' (Menzel, 2000; Chmielewski et al., 2004). 'Phenological phases were proposed by the European Environment Agency as climatic difference and global change indicators' (Menzel, 2003). Models of phenology are needed also for estimation of past climate conditions, improvement of primary productivity models, support of ecologists in biodiversity studies and bioclimatic zonations. 'The timing of phenological events is clearly correlated with different climatic factors including air temperature, soil temperature, precipitation, solar radiation, evapotranspiration, day length, snow cover etc.' (Wielgolaski, 2001). In mid and high latitudes, with a vegetation rest in winter and an active growing period in spring-summer time, plant phenology is mainly driven by temperature and photoperiod (Galan et al., 2001; Chmielewski et al. 2004). In many studies accumulated temperature is recognized as the main factor influencing year to year variation in phenology (Schaber, Badeck, 2003). 'It is also important to take amount of precipitation into consideration when studying pollen season, flowering, fruit production etc.' (Galan et al., 2001).'Phenology is broadly defined as the study of recurring plant and animal life cycle stages, especially their timing and relationships with weather and climate' (Schwartz, 2003). For example; fall of leaves, phehology date of leaves and flowers, the first appearance of butterflies, the first appearance of migratory birds and the date of egg laying of some animals are some of the phenological features. Most phenological phenomena are sensitive to changes such as temperature, light, wind, humidity. In the environment where every living thing lives, a variety of reactions and adaptations that vary from individual to individual are seen in parallel with climate change. Factors affecting plants such as winter frosts, late frosts, early autumn frosts, sunburn, drought, biotic and abiotic stress factors of plant species have been clarified by revealing the phenological characteristics of plants.

'In most disciplines, the term phenology is used interchangeably with seasonality, although the two are complementary in their description of ecosystem functioning; phenology referring to biotic processes, and seasonality referring to non-biological processes' (Friedl et al., 2006). The phenological characteristics of the species present in the selection of plants according to cultural and ecological conditions are taken into consideration. Observation of this situation provides advantages for producers. One of the branches of phenology is the determination of flowering dates in plants. The planting conditions of plants are related to some ecological, geographical conditions and genetic characteristics. Some plant taxa are bloom in warmer months due to genetic features; for example many Asteraceae taxa. On the contrary, *Veronica* taxa are mostly flowered in March-June (Öztürk, 2001).

There are many ways to collect and to analyze phenological data. Though considerable efforts are under way to improve this, until present there has been only little standardization on data collection – particularly across biomes and taxa. In terrestrial fields, relationships between various visible biological phenomena and meteorological/climate changes have been studied for a long time. Among these are flowering and leaf unfolding of plants, population dynamics and changes in community structures of insects, timing of oviposition and migration of birds, and so on. In addition to these studies, genetic and ontogenetic mechanisms driving these phenomena have been investigated recently (Vitasse et al., 2009; Aikawa et al., 2010; Kobayashi et al., 2013). In addition to such genetic factors, ecological, topographic, orographic, geographical and other conditions also play an important role in the flowering of plants.For example, *Robinia pseudoacacia* can bloom in the last week of May at the altitude of 1700-1800 m in Erzurum, which is located in the northern latitude, while at the same altitude in the southern latitude Van, it can bloom in the last week of May (Öztürk, 2010).

With this study, some plants in Van Yüzüncü Yıl University, Zeve Campus have been determined to provide phenological characteristics in Van ecological conditions and contribute to the related studies.

2. Material and methods

The identification of many plants were done made using 'Flora of Turkey' (Davis, 1965-1988) in the floristic work (Öztürk et al., 1998) that we have done in the field of Van Yüzüncü Yıl University Zeve Campus (Figure 1). Van Yüzüncü Yıl University Zeve Campus area is seen on Map 1. Many plants have been identified in the floristic work we had done before (Öztürk vd., 1998). Identification of some plants was also done using 'Flora of Turkey' (Davis, 1965-1985).

Fig. 1. Research area

During the vegetation development periods of 2013-2014, in the study area many flowering plant species bloomed dates were observed and recorded. Some of the most intense blooming dates have also been identified. Plant samples were collected, pressed and deposited in the VANF Herbarium. Studied plant taxa in this study were arranged according to the alphabetical order of the families. Some of the plant photos were given at the end of the text.

3. Results and discussion

The timing of flowering is one of the most widely investigated aspects of the phenology of plant life-cycles, and has been studied on every scale, from the level of the community (Mural, Sukumar, 1994) to that of the individual flower (Herrera, 1995). 'In most plant communities, although at least some species will be in flower throughout the growing season, there is a tendency for peaks of flowering to occur. In wet tropical forests flower production may coincide with peaks of irradiance' (Wright, Van Schaik, 1994). In the seasonally dry tropical forests flowering is often concentrated in the transition from the late dry to the early wet season (Murali, Sukumar, 1994). A particularly marked example of the concentration of community-wide flowering is found in the ground flora of temperate deciduous woodlands, where a pronounced peak of flowering occcurs in spring before the tree canopy closes (Heinrich, 1976). 'Flower production and maintenance requires the expenditure of energy to form non-photosynthetic tissue and nectar, the cost of which is considerable' (Ashman, Schoen, 1997). 'In many cases its timing may be largely determined by seasonal changes in resource availability. In some studies, climatic factors (such as some measure of cumulative heat sum) are the best predictors of flowering' (Diekmann, 1996; White, 1995). Smith-Ramírez & Armesto (1994) concluded from an investigation of a temperate rain forest in Chile that flowering (and fruiting) was largely constrained by seasonal variables. Also in this study flowering time of studied plant taxa were affected by seasonal and climatical variables.

In this study flowering period of 132 plant species belong to 33 families were determined. The plant list as follows.

GYMNOSPERMAE

PINACEAE

Pinus sylvestris L.: female cone formation 25.04.2014, 1.5 cm diameter; formation of male cone 10.05.2014, 1-2 cm diameter.

CUPRESSACEAE

Thuja occidentalis (cultivated): 07.05.2014.

Thuja orientalis (cultivated): Formation of male cone 10.03.2013; formation of female cone 10.04.2013.

Juniperus sabina L. (cultivated): Formation of male cone 28.04.2014; formation of female cone 28.04.2014.

ANGIOSPERMAE

APIACEAE

Echinophora tenuifolia L. subsp. *sibthorpiana* (Guss.) Tutin: 20. 07. 2014.
Pimpinella aurea DC.: 25. 04. 2014.

ASTERACEAE

Achillea vermicularis Trin.: 26.05.2014.
Achillea biebersteinii Afan: 15.05.2014.
Lactuca serriola L.: 20.07.2014.
Chondrilla juncea L. var. *juncea*: 24.07. 2014.
Tussilago farfara L.: 20.05.2013.
Tripleurospermum transcaucasicum (Manden.) Pobed.: 18.04.2014.
Taraxacum androssovii Schischkin: 01.04.2014.
Cnicus benedictus L. var. *benedictus*: 24.04.2014; 07.05.2014.
Tragopogon aureus Boiss.: 03.05.2013.
Centaurea depressa Bieb. : 02.05.2014, 10.05.2013. Most flowering time 22.05.2013.
Centaurea aggregata Fisch. et Mey. ex DC. subsp. *aggregata*: 23.06.2013; 15.07.2014.
Centaurea solstitialis L. subsp. *solstitialis*: 03.07.2013; 10.07.2014.
Centaurea balsamita Lam.: 03.07.2013.
Centaurea iberica Trev ex Sprengel: 15.07.2014, 29. 06. 2013.
Centaurea virgata Lam.: 08.07.2013.
Carduus nutans L. subsp. *leiophyllus*: 10.06.2014.
Xeranthemum annuum L.: 15.07.2014.
Anthemis wiedemanniana Fisch. et Mey: 05.05.2014.
Scorzonera suberosa K.Koch subsp. *suberosa*: 01.05.2014.
Scorzonera mollis Bieb. subsp. *mollis*: 22.04.2014.
Onopordum candidum Nab.: 01.07.2013.
Cichorium intybus L.: 06.07.2013 (most flowering time 07.07.2013).
Senecio aquaticus Hill. subsp. *erraticus*: 03.05.2013.
Senecio vernalis Waldst et Kit.: 16.04.2014.

ACERACEAE

Acer negundo L.: 15.05.2014.

BETULACEAE

Betula pendula Roth: 01.05.2014.

BERBERIDACEAE

Berberis vulgaris L.: 07.05.2013, 05. 05. 2014 (most flowering time 20.05.2013).

BORAGINACEAE

Buglossoides arvensis L. (Johnston): 17.04.2014.
Asperugo procumbens L.: 14.05.2014.
Anchusa aucheri L.: 18.04.2014.
A. azurea Mill. var. *azurea*: 16.04.2013, 15.04.2014 (most flowering time 1-25.05.2013).
Nonea pulla (L.) DC. subsp. *scabrisquamata* A. Baytop: 10.05.2013, 02.05.2014.
Alkanna orientalis L. (Boiss.) var. *orientalis*: 08.04.2014; 31.03.2013, most flowering time 10.04 - 03.05.2013.

BRASSICACEAE
Alyssum desertorum Stapf. var. *desertorum*: 20.05.2014.
Alyssum hirsutum Bieb. var. *hirsutum*: 10.05.2014.
Crambe orientalis L. var. *orientalis*: 13.05.2014.
Sisymbrium orentale L.: 02.05.2014; 27.04.2014.
Cardamine microphylla (Willd.)O.E.Schulz: 20.04.2014.
Brassica elongate Ehrh.: 01.04.2014.
Thlaspi perfoliatum L.: 22.04. 2013.
Thlaspi arvense L.: 20.03.2013.
Cardaria draba L. (Desv.) subsp. *draba*: 16.04.2013, most flowering time 10-15.05.2013.
Capsella bursa-pastoris L. (Medik).: 20.04.2014.
Raphanus raphanistrum L.: 15.04.2014.
Raphanus raphanoides: 17.04.2013.
Malcolmia africana (L.) R. BR: 20.05.2013.
CARYOPHYLLACEAE
Vaccaria pyramidata (Mill.) Rauschert: 05.06.2014.
Holesteum umbellatum L. var. *umbellatum*: 01.04.2014.
Holesteum umbellatum L. var. *tenerrimum* (Boiss.) Gay: 01.04.2014
CONVOLVULACEAE
Convolvulus arvensis L.: 25.05.2014.
GROSSULARIACEAE
Ribes rubrum L. (cultivated): 19.04.2014, 22.04. 2013
CAPRIFOLIACEAE
Tremastelma palaestinum (L.) Janchen: 08.07. 2013, most flowering time 13.07. 2013
ELAEAGNACEAE
*Elaeagnus angustifoli*a L.: Most flowering time 20-30.06.2014
EUPHORBIACEAE
Euphorbia terracina L.: 21.05.2013.
Euphorbia orientalis L.: 18.04.2014.
FABACEAE
Glycirrihiza glabra L. var. *glandulifera*: 25.05.2014.
Sophora alopecuroides L. var. *alopecuroides*: 01.06.2013.
Trifolium dubium Sibth.: 17.04.2014.
Trifolium pratense L. var. *patense* Boiss.et Bal.: 14.05.2014
Trifolium resupinatum L. var. *resupinatum*: 01.05.2014.
Astragalus chaldiranicus Kit. Tan et Sorger: 05.05.2014, most flowering time 16-20.05.2013.
Coronilla varia L. var. *varia* : 02.07.2013
Melilotus officinalis (L.) Desr.: 22.05.2013.
Onobrychis viciifolia Scop.: 20.05.2013.
Lathyrus aphaca L. var. *affinis* (Gus.) Arc.: 15.03.2013, 13.05.2014.
Robinia pseudoacacia L. (cultivated): 23.05.2013, 23.05.2014; most flowering time 1-15.07.2014.
Medicago sativa L. subsp. *sativa*: 15.05.2014.
GERANIACEAE
Geranium tuberosum L. subsp. *tuberosum*: 16.04.2013, 22.03.2014.
Geranium dissectum L.: 15.04.2014.
Erodium cicutarium (L.) La Herit. subsp. *cicutarium*: 20.04.2014.
IRIDACEAE
Gladiolus atroviolaceus Boiss.: 01.06.2014.
Iris germanica L. (k. b.): 16.06. 2014.
LAMIACEAE
Ziziphora persica Bunge: 15.05.2014.
Lamium purpureum L. var. *purpureum*: 01.04.2014.
Ballota nigra L. subsp. *nigra*: 01.06.2013.
LILIACEAE
Muscari neglectum Guss.: 10.03.2013, most flowering time 10-27.04.2013.

Gagea glacialis K.Koch: 01.04.2014.

PAPAVERACEAE

Papaver rhoeas L.: 04.05.2013, 06.05.2014, most flowering time 15-20.05.2013.

Glaucium corniculatum (L.) Rud. subsp. *corniculatum*: 07.06.2014.

PLANTAGINACEAE

Plantago atrata Hoppe: 28.05.2014.

Plantago lanceolata L.: 07.05.2014.

Plantago major L. subsp. *major*: 20.05.2013.

POACEAE

Poa pratensis L.: 24.04.2013, 18.04.2014.

Poa bulbosa L.: 18.04.2014.

Aegilops triuncialis L. subsp. *triuncialis*: 01.07.2014.

Bromus tectorum L. subsp. *tectorum*: 10.04.2014.

Hordeum violaceum Boiss. et Huet: 28.06.2014.

Elymus hispidus Opiz (Melderis) subsp. *hispidus*: 02.07.2014.

Eragrostis minor Host: 26.06.2014.

Eremopyrum distans (K.Koch) Nevski*:* 01.07.2014.

Taeniaterum caput-medusae subsp. *crinitum*: 05.05.2014.

Secale montanum Guss.: 16.06.2014.

Alopecurus myosuroides Hudson subsp. *myosuroides*: 15.06.2014.

Hordeum murinum L. subsp. *murinum*: 01.06.2014.

Phleum pratense L.: 15.07.2014

PRIMULACEAE

Androsace maxima L.: 09.04.2014.

RESEDACEAE

Reseda lutea L. var. *lutea*: 13.05.2013, 28.04.2014.

ROSACEAE

Malus floribunda (cultivated): 30.04.2014.

Malus domestica (cultivated): 01.05.2014.

Sanguisorba minor Scop. subsp. *minor*: 02.05.2014.

Rosa canina L. (cultivated): 16.05.2014.

Rosa sp. Pink layered rose (cultivated): 01.06.2014.

Rosa sp. Pale pink crowned rose with a crown (cultivated): 01.08. 2014.

Crateagus orientalis Pallax ex Bieb. subsp. *orientalis* k.b.: 10.05.2014, most flowering time 21-28.05.2014.

Cydonia vulgaris (cultivated): 20.05.2014.

SALICACEAE

Salix babylonica L. (cultivated): 10.04.2014.

Salix caprea L. (cultivated): 15.04. 2014.

Salix alba L. (cultivated): erkek çiçek durumları: 10.04.2014.

Salix viminalis L. (cultivated): 18.04.2014.

SCROPHULARIACEAE

Scrophularia carduchorum R.Mill.: 15.05.2014.

Veronica polita Fries.: 15.03.2014.

Veronica persica Poiret: 10.04.2013.

Veronica cymbalaria Bodard: 01.04.2013, most flowering time 5-20.04.2013.

Veronica triloba (Opaz) Kerner: 28.04.2013, most flowering time 05.05.2013.

IXIOLIRIACEAE

Ixiolirion tataricum subsp. *montanum*: 01.05.2014.

POLYGONACEAE

Rumex crispus L.: 05.05.2014.

RANUNULACEAE

Adonis flammea Jacq.: 08.05.2014.

Ceratocephala falcatus (L.) Pers.: 05.04.2013, most flowering time 20-25.04.2013.

Ranunculus cuneatus Boiss.: 10.04.2013.

Ranunculus arvensis L.: 01.04.2014.

RUBIACEAE
Callipeltis cucullaria (L.) Steven: 15.05.2014.
Galium tricornutum Dandy: 28.04.2014.
THYPHACEAE
Typha angustifolia L.: 15.07.2013

4. Conclusion

Plant phenology models are important tools in a wide range of issues such as agricultural practices, forestry, prediction of the impact of global warming, and aerobiology. Possibilities of predicting flowering time for wild and cultivated plants were studied based on meteorological and phenological variables in some part of Turkey. This work will shed light on the work on this topic and will help to keep these efforts easier and more productive. As a result of the research, new data on the floral phenologies of the studied taxa have been obtained. It is also important to enrich the VANF herbarium by making a herbarium sample of the collected plant specimens.

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We would like to thanks Yunus Emre Can Öztürk and Hüseyin EROĞLU, to help some parts of this paper.

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Appendix

Fig. 1. Some plant taxa in the research area
1-3: Zeve campus, 4: *Tamarix smyrnensis*, 5: *Erodium cicutarium* subsp. *cicutarium*, 6: *Aegilops triuncialis* subsp. *triuncialis*, 7: *Convolvulus arvensis*, 8-9: *Rosa* sp., 10: *Hordeum violaceum*, 11: *Eragrostis minor*, 12: *Althea officinalis*, 13: *Ribes rubrum*, 14: *Geranium tuberosum* subsp. *tuberosum*, 15: *Prunus* sp., 16: *Bromus tectorum* subsp. *tectorum*, 17: *Alyssum hirsutum* var. *hirsutum*,18: *Pinus sylvestris*, 19: *Betula pendula*, 20: *Picea orientalis*, 21: *Picea glauca*, 22: *Salix babylonica*, 23: *Sisymbrium orientale*, 24: *Ranunculus cuneatus*, 25: *Acer negundo*, 26: *Berberis vulgaris*, 27: *Alopecurus myosuroides*, 28: *Phleum pratense*, 29: *Syringa vulgaris*, 30: *Cnicus benedictus* var. *benedictus*, 31: *Juniperus sabina*, 32: *Trifolium resupinatum*, 33: *Trifolium pratense* var. *pratense*, 34: *Hordeum murinum* subsp. *murinum*, 35: *Muscari neglectum*, 36: *Veronica cymbalaria* 37: *Lotus corniculatus* subsp. *corniculatus*, 38: *Iris germanica*, 39: *Rumex crispus*, 40: *Onopordum candidum*.

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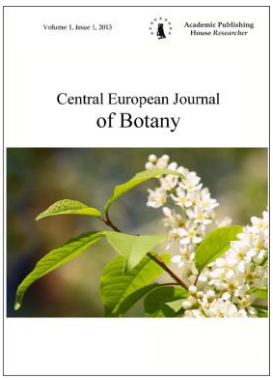

Reasons of Upgrading *Veronica anagalloides* subsp. *heureka* to Species Level as *Veronica heureka*

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Abstract

With plant samples related to *Veronica anagolloides* Guss. which was previously identified as *Veronica michauxii* Lam. by Öztürk and mentioned it's name in his doctoral theises and in a publication. Samples published by M.A.Fischer as *Veronica anagolloides* Guss. subsp. *heureka* M.A. Fischer, due to reasons stated in this article it needs to be raised to species level named *V. heureka* (M.A. Fischer ex) A.Öztürk & Ö.Kılıç. In an article cited samples were given abortive named as *V. heureka* by Öztürk & Fischer. Because of the impossibilities in 1977, Öztürk identified *Veronica anagolloides* as *Veronica michauxii* due to more morphological similarities. M.A. Fischer in his paper which published in 1981, because of the large number of transitional forms in terms of morphological characters and at the time of his impossibilities he had to classify at subpecies level named *Veronica anagolloides* subsp. *heureka*. In later years, karyological evidences were obtained and publications were made to support this classification. Their details are mentioned in this article text.

Keywords: Scrophulariaceae, Veronicaceae, *Veronica*, *V. anagalloides*, *V. heureka*, stat. n.

1. Introduction

Veronica L. is a large diverse genus of the Veronicaceae sensu Angiosperm Phylogeny Group with approximately 450 species in the world. It's taxa distributed mainly in the temperate regions of the northern hemisphere and Australia (Albach et al., 2004). In Flora of Turkey *Veronica* taxa are generally annual or perennial; leaves are facing one another; divided or undivided; flowers are in racemose or spica state; corolla is round, slightly zygomorphic, bluish, purple, reddish and in oviform; fruits are bilocular, locular or in septicidal capsule form; seed are in high and low numbers and in puffed or variolitic form (Fischer, 1978). *Veronica* has high species number and has more than 250 species around the world; 86 species and more than 107 taxa were found to be in Turkey (Öztürk, 1977; Öztürk, 2001; Albach, Chase, 2001).

In the past years, identification and classification of *Veronica heureka* hardship by Fischer and Öztürk. Öztürk investigated this species by morphological and compared with literature samples which was collected from Allahuekber mountains in 1976, to determined it is a new species or not. In 1977 Öztürk was identified and specified this species as *Veronica michauxi* Lam. in his PhD theises, because of more morphologic similarities. In 1978 Öztürk took these samples to Vienna and showed them to Fischer. When Fischer saw these samples in the Öztürk's PhD theises

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(Öztürk, 1977), he declared that he studied to clear this statement for five years and now he has cleared this statement. Then he used '*heureka*' latin word which means 'I found'. Then Fischer identified these samples as *V. anagalloides* subsp. *heureka* M.A. Fischer in'Flora of Iranica'. This decision of Fischer was influenced by incomplete examinations and transitional forms at that time. In later years, as a result of karyosistematic and karyological examinations, this issue has become more enlightened (Öztürk, Fischer, 1982; Fischer, 1982; Öztürk, Öztürk, 2000). During these investigations, some important morphological and karyological differences were found and compared. Thus, it has been concluded that the specimens should be a separate species.

In the light of these studies, it has become necessary to raise the *V. anagalloides* Guss. subsp. *heureka* M.A.Fischer to new species level as *V. heureka* (M.A. Fisch.) ex A.Öztürk & Ö. Kılıç.

2. Relevance

In this research, we proved that *Veronica heureka* (M.A. Fisch.) ex A. Öztürk & Ö. Kılıç should be evaluated as a separate species, because of habitus, morphological, karyological and karyosystemic findings.

3. Material and methods

To reach the target of this article we used figures (Figures 1-5), datas and literature sources about *Veronica* taxa (Öztürk, 1978; Öztürk, 1983; Öztürk, 1982; Davis, 1978; Elçi, 1994; Darlington, 1976; Öztürk, 2006; Öztürk, 2008) and plant materials of *Veronica* taxa are deposited herbarium of Van Yüzüncü Yıl University (VANF).

General view of *Veronica* taxa is seen in Figure 1.

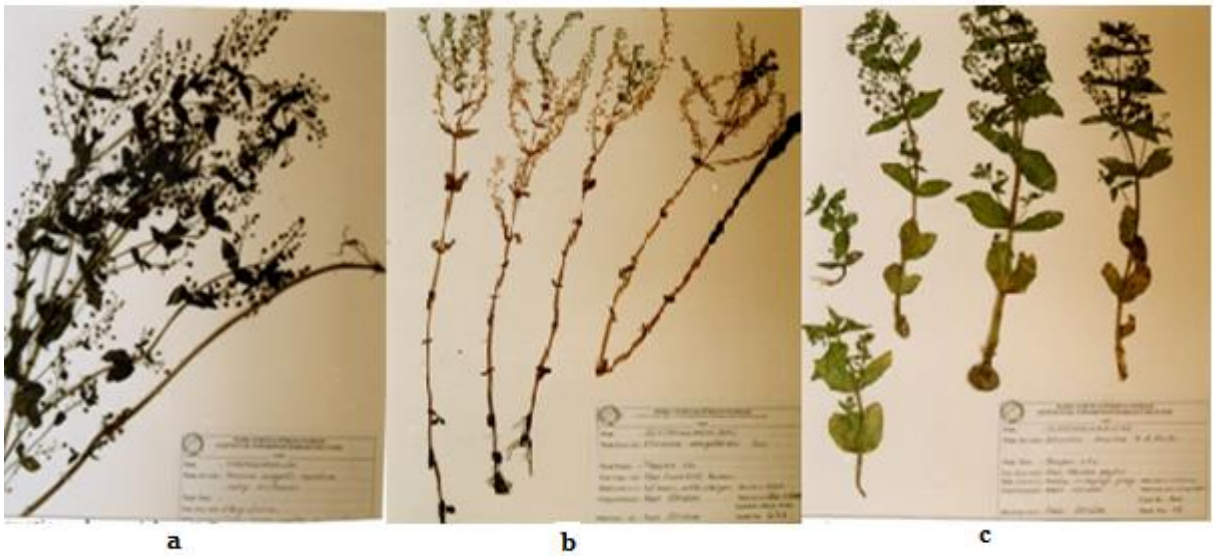

Fig. 1. General view of **a:** *V. anagallis-aquatica* subsp. *michauxi*, **b:** *V. anagalloides*, **c:** *V. heureka*

4. Results and Discussion

According to obtained results, the upgrade of *Veronica anagalloides* subsp. *heureka* to species level with the name *Veronica heureka* is due to the morphological and karyological differences between these taxa. In 1981 at Flora of Iranica *V. anagalloides* subsp. *heureka* published by Fischer and Öztürk; then Fischer mentioned these population samples as *V. heureka* in 1991 (Öztürk, Fischer, 1991). There are significant morphological and karyological differences when compared to the population samples of *V. anagalloides* subsp. *heureka* and *V. heureka*. Although the leaf blade is 5-30 x 15-60 mm in *V. heureka*, in *V. anagalloides* leaf blade is 2-10 x 25-40 mm. Details of these differences are shown in Table 1.

Table 1. Character Encounter Table

Characters	*Veronica anagalloides* subsp. *heureka*	*Veronica heureka*
Shape of leaf	Linear-lanceolat	Eliptic ovat-lanceolat
Leaf size	7-20 x 3-10 mm	(8-)15-40(-60) x 10-25 mm
Leaf base	Sessil, not sieged the stem	Sessil, sieged the stem
Pedicel connecting angle to stem	(10°-) 20°-30°	(60°-) 70°-80° (-90°)
Capsul shape	Eliptic triangular	Orbicular, obovat
Total genom	31.372 µm	40.198 µm
Avarage chromosom length	1.743 µm	2.223 µm
Status of petals length	Narrow and capsuls are equal length	Wide and exerted capsuls
Petal/pedicel	½	1/1
Corolla colour	Bright blue	Pink, rose red, white-blue
Satellite	There are 1-2 satellit	There aren't satellit
Chromozom size	(2n) = 18	(2n) = 18
Corolla fauces	Glandular, fragrant	Glandular, fragrant

Obtained karyosistematic studies, distinctive chromosome size and morphological differences require raised this subsp. to species level (Tables 1-4, Figures 1-5).

Table 2. Chromosome measurements *V. anagallis-aquatica* subsp. *michauxi*

Chromosome No	Average chromosom length (µm)	Proportional length
1	4.755 ± 0.223	15,782
2	4.044 ± 0,236	13.422
3	3.666 ± 0.217	12.167
4	3.600 ± 0.240	11.948
5	3,333 ± 0,186	11.062
6	3,200 ± 0.151	10.621
7	2.733 ± 0.143	9.071
8	2.577 ± 0.117	8.523
9	2.222 ± 0.111	7.375

Total chromosome length: 60.260 (µm), **Average chromosome length:** 3.347 (µm)

Table 3. Chromosome measurements *V. anagalloides* subsp. *heureka*

Chromosome No	Average chromosom length (µm)	Proportional length
1	2.911 ± 0.312	18.558
2	2.533 ± 0.315 –SAT	16.148
3	2.033 ± 0.293	12.961
4	1.833 ± 0.286	11.686
5	1.577 ± 0.245	10.054

6	1.411 ± 0.217	8.995
7	1.333 ± 0.236	8.498
8	1.133 ± 0.180	7.223
9	0.922 ± 0.167	5.878

Total chromosome length: 31.372 (µm), **Average chromosome length:** 1.743 (µm)

Table 4. Chromosome measurements *V. heureka*

Chromosome No	Average chromosom length (µm)	Proportional length
1	3.144 ± 0.464	15.643
2	2.667 ± 0.373	13.269
3	2.522 ± 0.254	12.548
4	2.333 ± 0.217	11.608
5	2.244 ± 0.206	11.165
6	2.167 ± 0.218	10.782
7	2.033 ± 0.163	10.115
8	1.922 ± 0.168	9.563
9	1.067 ± 0.142	5.309

Total chromosome length: 40.198 (µm), **Average chromosome length:** 2.233 (µm)

In a research about chromosom and caryotype analayzes of *V. anagalloides* populations, two pairs of satellite have been detected on two chromosomes (Öztürk, Fischer, 1982).

In another study, 1 satellite was detected in 2 chromosomes (Öztürk, Öztürk, 2000). Despite the presence of satellites in the chromosomes of the populations of *V. anagalloides*, no satellite could be detected in the chromosomes of the populations of *V. heureka*.

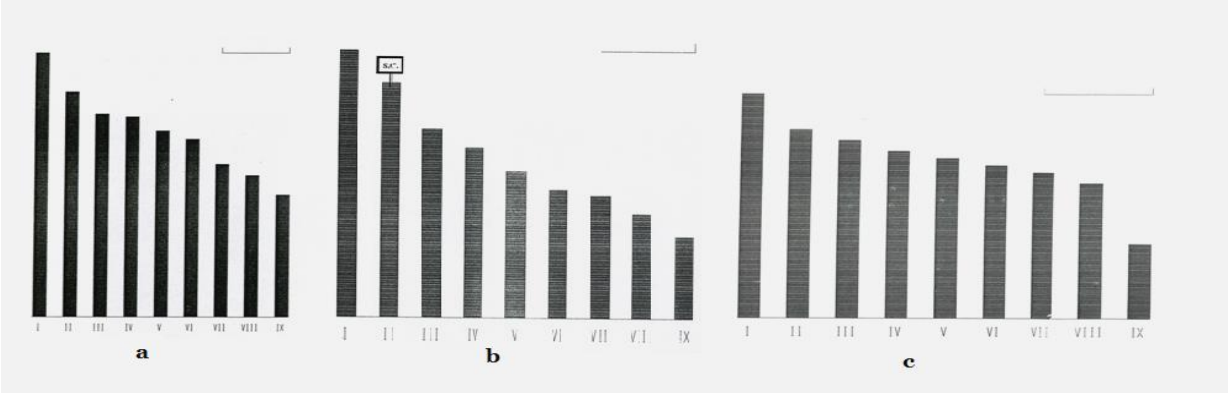

Fig. 2. Idiograms (Bar 1µm) of **a:** *V. anagallis-aquatica* subsp. *michauxi*, **b:** *V. anagalloides*, **c:** *V. heureka*

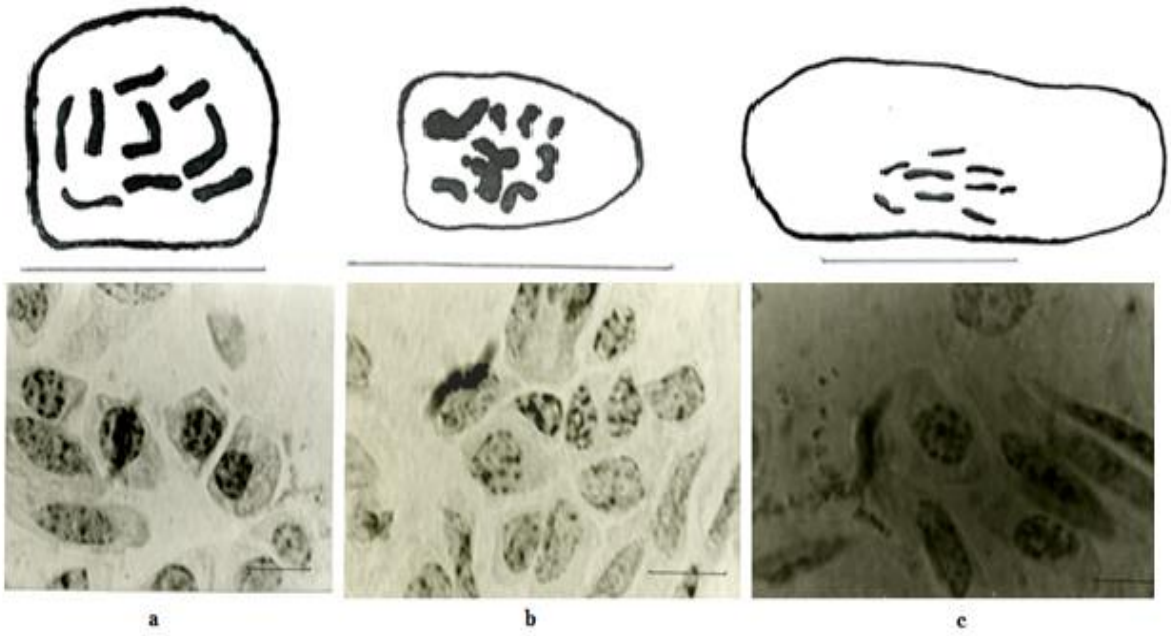

Fig. 3. Meiotic metaphase chromosomes in pollen mother cell (Bar 10 μm) of **a:** *V. anagallis-aquatica* subsp. *michauxi*, **b:** *V. anagalloides*, **c:** *V. heureka*

In *V. anagalloides*, pedicel are stand out from raceme axes narrow angle (20-30°) but in *V. heureka* pedicel are stand out from raceme axes vertical angle (80-90°). Nevertheless in *V. anagalloides* the capsule is in a narrow elliptical structure, in *V. heureka* base of capsule is wide and orbicular-rounded shape (Figure 4).

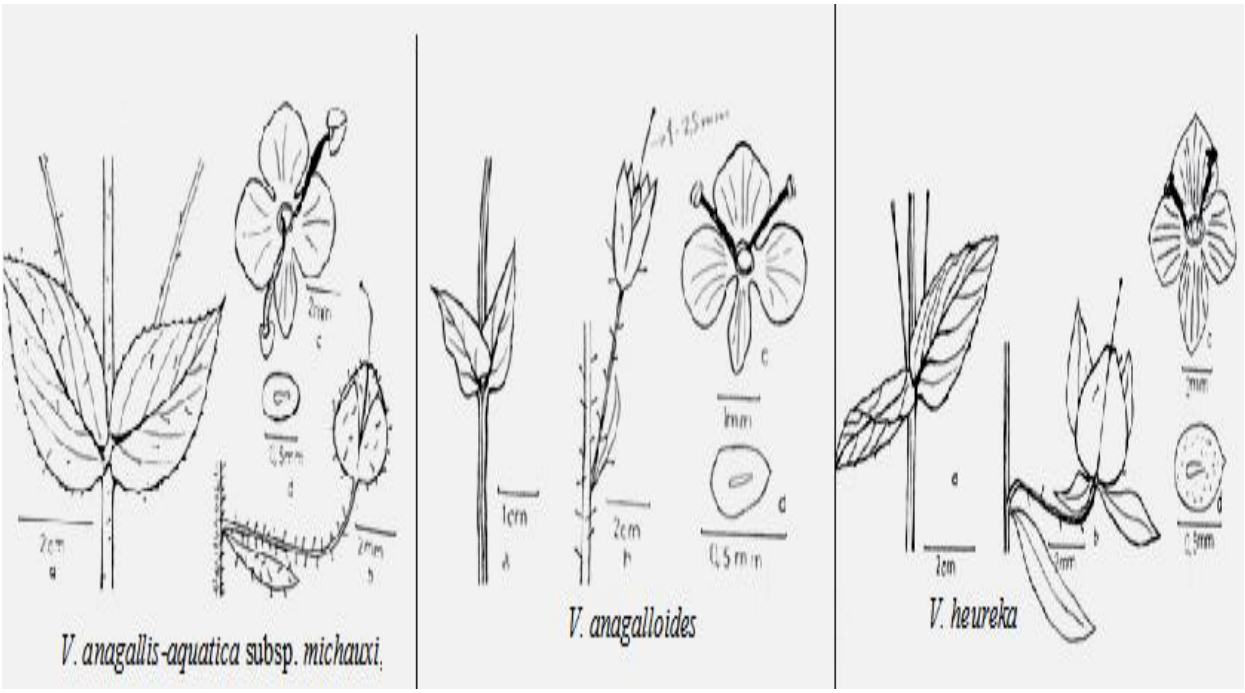

Fig. 4. a: stem and leaves; **b:** fruiting pedicel, peduncul, capsul and stilüs;
c: corolla and stamens; **d:** seed

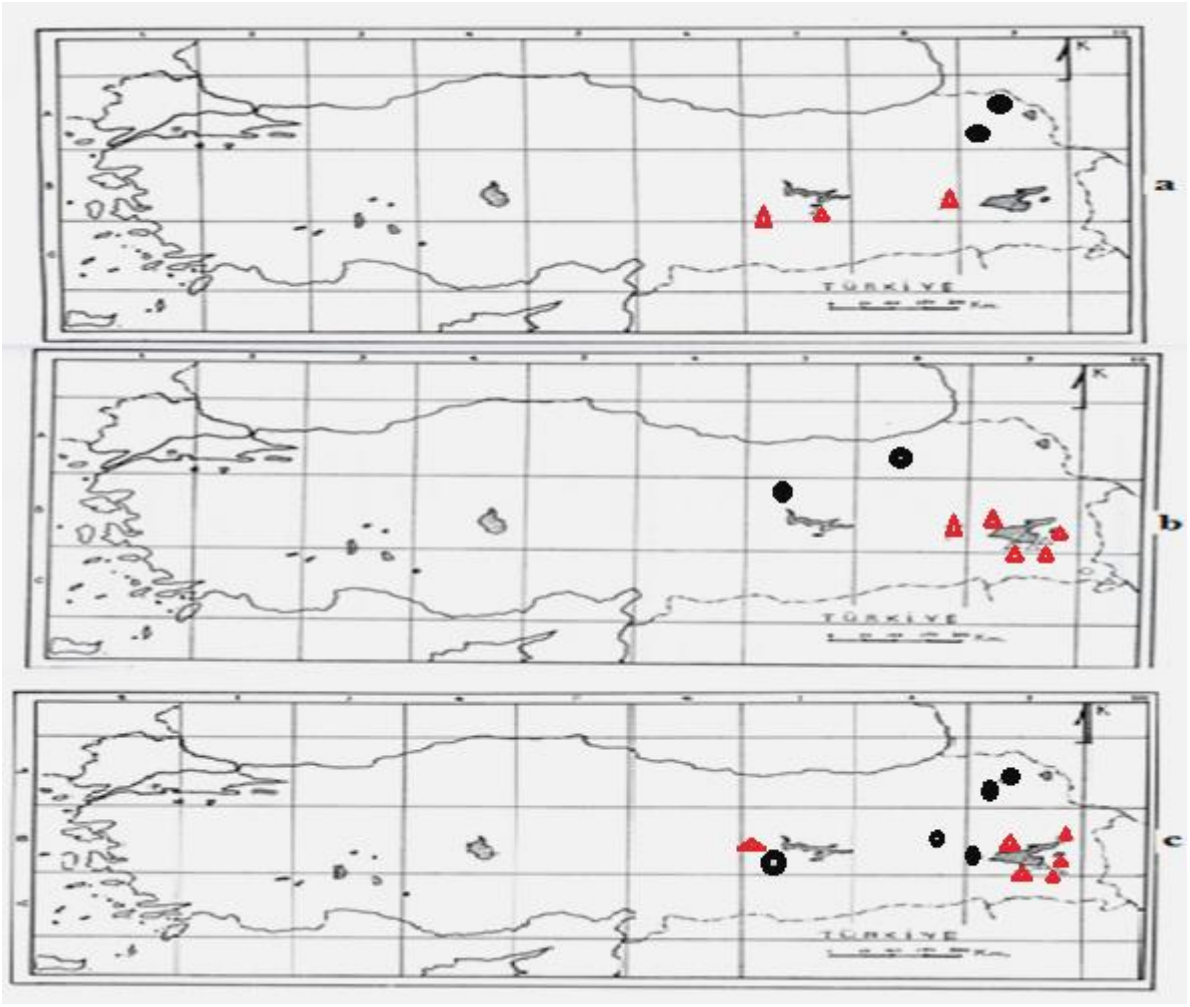

Fig. 5. ▲new localities, ●previous localities of **a:** *V. anagallis-aquatica* subsp. *michauxi*, **b:** *V. anagalloides*, **c:** *V. heureka*

When other distinctive morphological differences (Table 1) added, it is a scientific necessity to increase the previously mentioned subsp. to the species level.

5. Conclusion

According to the evaluations made in light of the new scientific findings and determinations mentioned in this article, it is appropriate to classify the problematic taxon as a separate taxon from *V. anagalloides* and to raise it to species level as *V. heureka* (M.A. Fischer ex) A. Öztürk & Ö. Kılıç, stat. nova.

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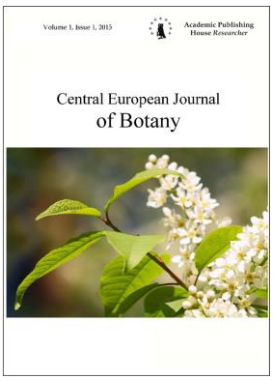

Bio-Ecology of Some Coniferales Introduced in Eastern Georgia

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Abstract

The article discusses the introduction results of two genera species of Pinaceae family, genus pinus and genus picea, introduced in Eastern Georgia.

We have studied the periods of bud opening, vegetation ending, starting and finishing of cambium action, sprout woodening process, time and rate of growing in height, and regularities of accumulation-transformation of storage carbohydrates.

The studies revealed that the annual development cycle of all these species includes all morphological-physiological periods: sprout growth, latent growth, organic and forced rest. They are characterized by the good growth-development; almost all of them are perspective for eastern Georgia, particularly, for all regions of inner Kakheti.

However, in recent years, massive drying up of pine forests groves takes place in eastern Georgia. That is why it is necessary to conduct the fitopatologic research, identify the pest causing the damage and plan the measures against it.

Keywords: Pinus, Picea, vegetation, cambium, life expanse, height growth period. accumulation-transformation of carbohydrates.

1. Introduction

Introduction of new plants in Georgia has a long history. About 2500 species of introduced woody plants exist here. Almost all of them belong to ecological group. We have studied the results of acclimatization-adaptation of two genera species of Pinaceae family, genus **pinus** and genus **picea**, introduced in Eastern Georgia.

From the gymnosperm of modern flora, coniferous plants are the richest in representatives. Genus Pinus L. involves approximately 100 species, naturally spread in the North Hemisphere. More than 40 species are introduced in Georgia.

Genus Picea includes about 45 species, naturally spread in temperate and cold zones of the north Hemispheres. Only one species is spread wildly in Georgia – **Picea orientalis Link**.

We have studied the bio-ecology of the following species of Genus **Pinus and Genus Picea** spread in eastern Georgia. **Picea abies Karst., Picea alba Britt, Picea pungens Engelm., Picea morinda Link. Pinus eldarica, P. Griffithii, P. cembra, P. Pallasian, P. pinea, P. sabiniana, P. silvestris, P. sosnovskyi, P. Strobus.**

2. Materials and methods

The aim of our research was the study of bio-ecological peculiarities of the listed species of

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genus Pinus and Picea introduced in Georgia; summarizing the issues of their introduction-acclimatization. For this reason, the observation has been conducted on the selected specimens since 2000. The penology and penometry has been conducted on the selected plants systematically, once or twice in the decade. We have observed the periods of bud opening, vegetation ending, starting and finishing of cambium action, sprout woodening process, the time and rate of the height growth.

Besides the apical growth, the cambial growth of the lateral branches has also been studied. For this purpose we took the patterns from the twigs once in every ten days from the early spring to the late autumn, then placed them in 60-70 % alcohol (Yatsenko, Chmielewski, 1954; Lobzhanidze, 1961; Tsitsvidze, 1973), and observed the dividing of the cells of secondary meristem and development of annual cycle through a microscope. Transverse veneer was taken with safranin and the process of new wood cells development was determined; we conducted the research according to the methodical instructions by Yatsenko-Khmelevski, 1954, Lobzhanidze E., 1961, and Tsitsvidze A., 1973.

We have also studied the peculiarities of accumulation and transformation of storage carbohydrates in the lateral branches with regard to annual development rhythm and overcoming winter frosts. For this purpose in the third decade of each month, the analyzing samples were taken every morning. By the influence of chemicals on the diametrical slices, we studied carbohydrate content. Starch content was determined by the Potassium iodide (starch stained in blue), sugar – by alpha-naphthol, and concentrated sulfuric acid (stained in purple), and fats with the help of Sudan III (color – orange) (Japaridze, 1953). We used a five-point system (1 – very small, 2 – small, 3 –satisfactory, 4 – much, 5 – too much).

3. Results

The research revealed that under the conditions of eastern Georgian, the studied species begin growth in spring, when the average daily temperature is 5-10°, and end at different times, depending of the endogenic and exogenic factors, in May-June in lateral and in June-July – in top branches.

Besides, we have noticed that on Pinus griffithii and Pinus strobus, the growth is more intensive at night, than in the day. It mostly refers to the sprout lengthening. For example, for 10 days from April 20th to April 30th the growth of Pinus griffithii and Pinus strobus during the night was 2,5 cm and during the day – 1,8cm. In addition, revealed that the sprout growth is not continuous, resting period 2-3 days, rarely – 4days, after about 8-15 days of growing.

Pinus eldarica is very perspective for eastern Georgian conditions, so in this region, there are more than hundred hectares of cultivated forests with Pinus eldarica. Almost all forms are used in greenery of parks and gardens, street plantations, road greenery, alley formation, etc. Pinus eldarica forms a well-developed trunk, fruit-bears abundantly, and in most cases, reproduces by self-sowing. However, in recent years, trees in groups or individually are drying. In our opinion, this may be caused by the breach of water balance in soils or humidity, and pests. Therefore, we believe that it would be reasonable to cultivate it not in pure (only Pinus eldarica) but in forms of mixed plantation.

Seed fullness of Pinus cembra is 3-5 %. Such a low quality is because the cold zone plants require long-term effect of low temperature for normal development of generative organs. That is why; lime content in soil, short days and relatively warm winter is restrictive for their growth-development here. Therefore, this species is almost useless for greenery and foresting of eastern Georgian regions (in oak belt and lower).

Pinus pallasiana begins the bud opening in the first decade of April. The growth duration is 60-65 days; cambium activity period – 150-160 days, seed fullness – 60-80 %. Two peaks of maximal starch content in the branches were observed in summer, and relatively lower- in August-September. Starch content decreased in January, when the air temperature equaled – 9,5°. At this time, fat content was maximal and equaled 5 points. Sugar content increased from autumn to January and in February, it decreased to 2 points from 3.

Pinus pallasiana is distinguished by the good growth and development in Tsinandali Park, as well as in other regions of Kakheti. Therefore, it is widely cultivated throughout Kakheti. In Telavi Pinus pallasiana culture is artificially cultivated on approximately 6 hectares.

At the age of 30-40 Pinus sosnovskyi is 15-18m in height; stem diameter is 28-30cm; fruit-bears regularly (seed fullness is 80-90 %) and reproduces by self-sowing as well.

Well-developed specimens of Pinus pinea exist in Tbilisi, as well as in Tsinandali Park, where two of its centenarian trees are characterized with good growth-development (diameter – 75-88 cm, height – 18-20 m). Pinus pinea is perspective for eastern Georgian regions and from the decorative viewpoint, great attention should be paid to its widespread planting in greenery.

At the age of 70, Pinus sabiniana reaches 16m in height and 50cm in diameter in Tsinandali Park. It fruit-bears abundantly and hardly ever dies from droughts. This species loves light, does not stand against darkening, slightly demanding to the soils, adapts well to grey-brown soils of neutral and weak alkaline reactions; is not damaged by the frosts.

In Tsinandali Park, 59-60 years old Pinus silvestris grows up to 20m and 30-32cm diameter; pollinates in late April and mid-May. In eastern Georgia, it is hardly ever cultivated. It loves light, cannot stand darkening and saline soils.

Various forest cultures of Pinus sosnovskyi covers several hundred hectares in eastern Georgia and has been included in the artificial forest cultivation assortment for a long time. 30-40 years old specimens, existing in Telavi, are 15-18m in height, 28-30cm in stem diameter, fruit-bear regularly (seed fullness -80-90 %) and reproduce by self-sowing as well.

Pinus strobus is characterized with a good growth-development in Tbilisi, Telavi, Tsinandali Park. About 90-100 years old specimen reaches 20-21m in height and 70-75 cm in width; fruit bears abundantly; seed fullness is 30-40 %; sometimes provides self-sowing as well. It begins growing in May. Growth duration is 40-45 days; duration of cambium activity – 145-150 days. It grows so well in Kakheti that may be widely cultivated in decorative gardening and forest cultures.

Picea abies and P. pungens start growing in May and last approximately 33-39 days. Picea abies is characterized with withered crown.

The growth of Picea orientalis and P. Pungens starts in April-May and finishes in June, or rarely in July or August and lasts for 46-70 days. They are characterized with withered crown.

The maximal amount of starch in the branches of Picea excels Link is in April, and the maximal amount of fats is from October to February. Sugar content increases from the end of September, perhaps due to the day shortening and the fall of temperature to 5-8^{0}, i.e. preparation for winter is presented as a hereditary sign.

4. Conclusion

The researches revealed that endogenous rhythm of the studied plants is well adapted to the climate of eastern Georgia and isn't damaged by the frost, grows well and gives the seed able to rise. All of them are very interesting plants and it's preferable to use them widely in the parks and gardens of eastern Georgia.

The regularity of accumulation-transformation of carbohydrates correlates to the annual development cycle. Maximal starch content was revealed in autumn – September-October and in spring –April-May. Starch transformation into sugar begins in autumn, before the frosts.

However, despite above mentioned, during the last 6 years reddening of needles, young sprout drying, stem damage by pests in artificial and natural groves of pine forests is observed in all regions of eastern Georgia. During the last 2 years, massive drying of pine groves takes place.

We suggest conducting fitopatologic research, identifying the pests causing the damage and planning the measures against them.

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