

DROUGHT STRESS IN CEREALS – A REVIEW

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Drought is one of the most important factors that influences plant morphology, biochemistry, and physiology, and finally leads to the decline in crops productivity and seed quality. Climate change, severe changes in water availability together with thermal stresses environment coincide with increasing human population, and to reveal sustainable solutions it is necessary to understand: i) how cereals react to drought, ii) how the tolerance mechanisms are exhibited by the genotype, and iii) which approaches enable to increase the tolerance of crop species against limited water availability. Especially in cereals as in high-quality food sources, it is important to reveal the adaptation mechanisms to rainfall dynamics on arable land and to the prolonged period of drought. This review summarizes current knowledge on the impact of drought on cereals, the mechanisms these crops utilize to cope water scarcity and survive, and the most efficient approaches to improve their drought tolerance.

Key words: drought, wheat, dehydrins, abscisic acid, reactive oxygen species, photosynthesis

1. INTRODUCTION

Plants live in a constantly changing environment that often affects their growth and development. They adapt to life under stress conditions by adjusting their physiology, morphology, or metabolism to minimize stress-induced damages. Changes to the plant's biochemical and physiological processes ultimately causing damage to death are considered to plant stress (Atkinson & Urwin 2012). Drought, salinity, and temperature are the major environmental factors, which affect the geographical distribution and productivity of plants (Wang *et al.* 2003; Saradadevi *et al.* 2017; Siddiqui *et al.* 2017; Ghatak *et*

al. 2017), quality in agriculture and threaten food (Fedoroff *et al.* 2010; Parkash & Singh 2020).

The impact of abiotic stress continues to have dominant effects on crop production (Lobell *et al.* 2011), whereby only 3.5% of the total land area on Earth is unaffected by environmental factors (FAO 2013). Studies have shown that over the next 30 years, the Earth's average surface temperature will increase by about 0.2°C over a decade. It is estimated that the increase in average global temperature will increase by 2.5 to 4.5°C on average by the end of the 21st century due to increasing concentrations of greenhouse gases (such as CO₂, N₂O, and CH₄) in the atmosphere (Wang *et al.* 2018). The Food and

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Agriculture Organization (FAO) reported in 2018 that 83% of the damage and loss in agriculture as a share of total damage and loss across all sectors in years 2006–2016 was caused by drought (FAO 2018).

Most the plants have very little ability to cope with the environmental conditions in a short term and are extremely vulnerable to acute water shortages.

Wheat (*Triticum aestivum* L.) as the world's most important cereal crop is grown in a large range of latitudes worldwide under both irrigated and rain-fed conditions and thus in conditions subjected to drought. Wheat is considered as an excellent system to study drought tolerance in spite of its genetic complexity (Cheng *et al.* 2016).

2. PHYSIOLOGICAL CHANGES IN CEREALS DURING DROUGHT

Water scarcity occurs during drought, where water is simply not present in the soil. Physiological drought is not necessarily caused only by a lack of water in the soil, but it can also occur when there is an excess of water (the soil water potential is lower than in the plant) or high soil salinity (ions also affect the water potential). Thus, physiological drought is a condition where the plant cannot receive water (Lisar *et al.* 2012; Osakabe *et al.* 2014). The responses of plants to water stress are diverse and may involve the contribution of various defence mechanisms or modification of physiology, morphology, anatomy, biochemistry, as well as short and long-term developmental and growth-related adaptation processes (Abobatta 2019).

Physiological responses to drought and heat stresses providing an escape to the water or heat stress comprise morphological and physiological adjustments (Lamaoui *et al.* 2018). Enlarged root system (Gregorová *et al.* 2015; Abobatta 2019), reduced stomatal number and conductance, decreased leaf area, increased leaf thickness, and leaf rolling or folding (Earl & Davis 2003) to lessen evapotranspiration (Anjum *et al.* 2011; Lamaoui *et al.* 2018; Kapoor *et al.* 2020) are strictly associated with an adaptive response. Reduced plant growth and productivity under drought are caused by altered plant

water relations, decreased CO₂ assimilation, cellular oxidative stress, membrane damage of affected tissues, and in some cases, inhibition of enzymes activity. Under drought conditions, plants can alter water relations to maintain cellular functions. For example, plants exhibit osmotic adjustment by synthesizing and accumulating compatible solutes such as free amino acids, sugars, and proline (edit some references) (Izanloo *et al.* 2008; Tatar & Gevrek 2008). Osmotic adjustment allows the plant to maintain turgor pressure and cell volume at low water potential which is important for maintaining metabolic functions. In addition, osmotic adjustment facilitates the recovery of metabolic activities after relief from stress (Izanloo *et al.* 2008).

Although investigations have been made to study the recovery of photosynthesis from drought stress (Osakabe *et al.* 2014) in different crop species including wheat, studies addressing membrane stability, oxidative stress, antioxidative process, and osmolyte dynamics during drought recovery are limited. Studies quantifying the impact of plant metabolic changes during drought indicate, that stress conditions during vegetative growth periods can significantly influence grain yield of wheat (Araus *et al.* 2002; Souza *et al.* 2004; Siddiqui *et al.* 2017). After drought stress is removed, the availability of even a small amount of rainfall can have a significant effect on plant physiological functions, ranging from whole-plant physiological and morphological responses to biochemical responses (Abobatta 2019). Therefore, it is of particular importance to investigate the underlying mechanisms contributing to drought tolerance (Izanloo *et al.* 2008).

Water deficit in plants decreases or suppresses the process of photosynthesis (Earl & Davis 2003; Anjum *et al.* 2011; Kapoor *et al.* 2020). The decrease in the photosynthetic process under drought is mainly attributable to the decline in CO₂ conductance via stomata and mesophyll limitations, however, the decrease in photosynthetic activity due to drought may also be due to reduced ability of stomatal movement (Abid *et al.* 2018). The loss of CO₂ uptake affects Rubisco activity and decrease the function of nitrate reductase and sucrose phosphate synthase and the ability for ribulose biphosphate (RuBP) production (Singh & Thakur 2018).

The chlorophyll content is another photosyn-

thetic attribute strongly influenced by water deficit (Gregorová *et al.* 2015; Alghbari & Iksan 2018). For example, leaf chlorophyll synthesis and chlorophyll *a/b* proportion is altered by drought stress. A decline in photosynthetic activity, amount of chlorophylls, loss of photosystem II photochemical efficiency, alteration in stomatal movement, and disturbance in the water status of plants resulted in declined plant productivity. Among others, a major cause for the decline in the amount of chlorophyll due to drought stress is the drought-promoted O_2^- and H_2O_2 production, which results in lipid peroxidation and significant chlorophyll degradation. Drought stress also causes a reduction in the abundance of several Calvin cycle proteins, including Rubisco (Anjum *et al.* 2011).

In terms of metabolites, a reduced rate of photosynthesis disrupts carbohydrate production, lowers the level of sucrose in leaves, and prevents the transport of sucrose into organs. Ultimately, reproductive development is limited. In addition, drought induces vacuolar inverse of sucrose mediated by hydrolysis and modulation of osmotic potential. Drought inhibits the cell division of the developing embryo/endosperm resulting in the poor intensity of cell division and ultimately leading to germ abortion (Andersen *et al.* 2002).

2.1 Effect of drought on plant growth

Drought stress is well recognized as a limiting factor that alters multiple aspects of plant growth and development (Alghbari & Iksan 2018; Kapoor *et al.* 2020). Germination of seeds, health, coleoptile length, and leaf area are foremost for the plant progression. Seed germination is the primary aspect of growth that is sensitive to drought (Kapoor *et al.* 2020). Visible symptoms of the plant exposed to water scarcity in the initial vegetative stage is besides seed germination reduction also leaf wilting. Plant growth is also in term of reducing shoot length and fresh weight of the hypocotyls negatively affected by lack of water (Abobatta 2019). The interruption in establishment of buds and flowers is also observed due to a lack of nutrients from the dried soil (Abobatta 2019; Kapoor *et al.* 2020). The root system is the main plant organ for adaptation to drought stress conditions. In conditions of water deficit, plants seek to extract water from deeper

soil layers by boosting their root architecture (Mitra 2001; Lisar *et al.* 2012; Abobatta 2019). The main basis of variation appears to be constitutive, therefore, a root system architecture that allows reserve of more water quantity is the most important tool for drought tolerance (Abobatta 2019). Moreover, water availability is primarily recognized by roots, which in turn regulates its growth (Kudoyarova *et al.* 2011).

The plant leaf is an important part of the plant because of the process of photosynthesis, which in turn is the main driver of plant growth. Decrease in leaf area is a drought avoidance strategy because declining leaf area results in a decreased water loss by the process of transpiration (Earl & Davis 2003; Anjum *et al.* 2011; Kapoor *et al.* 2020) and this reduction in leaf area is attributable to the inhibition of leaf expansion by the declined rate of cell division, which results in loss of cell turgidity (Xu *et al.* 2010).

Abiotic stress will typically cause upper and lower spikelets and distal florets to either abort or produce small grains (Nuttall *et al.* 2017). A reduction in grain filling occurs due to a reduction in the assimilate partitioning and activities of sucrose and starch synthesis enzymes (Farooq *et al.* 2009), however, the period and duration of the stress factor is important. Following heading, drought had little effect on the rate of kernel filling in wheat, but its duration (time from fertilisation to maturity) was shortened, and dry weight reduced at maturity (Wardlaw & Willenbrink 2000). In barley, drought stress reduces grain yield by reducing the number of tillers, spikes, grains per plant, and individual grain weight. Post-anthesis drought stress was detrimental to grain yield regardless of the stress severity (Samarah 2005; Alghbari & Iksan 2018). In durum wheat, thousand-grain weight, grain protein yield, and test weight reduced significantly under both salinity and drought stress conditions, whereby salinity stress was greater than drought stress (Houshmand *et al.* 2014).

Soil water deficit condition reduces crop yield by reducing the plant growth according to the following three main mechanisms: i) reduction in canopy absorption of photosynthetically active radiation, ii) decreased radiation use efficiency, and iii) decreased harvest index (Earl & Davis 2003).

2.2 Effect of drought on seed quality

Drought stress leads to the yield losses of major crops worldwide every year (Wang *et al.* 2003; Saradadevi *et al.* 2017; Siddiqui *et al.* 2017). Reducing the availability of water during drought results in a reduction in total nutrient intake and nutrients concentration in plant tissues. A significant impact of the water deficit is manifested in the transport from the perception point, namely from the root system to the above-ground parts (Garg 2003). In general, drought stress induces an increase in nitrogen content (Li *et al.* 2013; Alghabari *et al.* 2018; Magallanes-López *et al.* 2017; Kapoor *et al.* 2020), a definite decrease in phosphorus levels, and does not have significant end effects on potassium content (Garg 2003). During booting and anthesis stages in winter wheat cultivation in a pot experiment under drought stress the concentration of nitrogen and sulphur were observed higher for dwarf cultivars, whereas no significant differences were observed between tall and semi-dwarf wheat cultivars (Alghabari *et al.* 2015).

Starch synthesis is highly sensitive to high temperature and drought stress. Its accumulation in wheat grains can be reduced by over 30% after heat treatment, at temperatures between 30°C and 40°C. Thus, the ability to synthesize, store, and remobilize starch at high temperature is crucial for the determination of grain sink strength (Ni *et al.* 2018). The effects of abiotic stress on the dietary fibre content of wheat and barley grains appear to be variable as one report suggests that β -glucan content of barley decreases under high temperature and drought stress (Savin *et al.* 1997), whereas another reports an increase in β -glucan content under drought stress (Jansen *et al.* 2013).

In durum wheat (*Triticum turgidum* L. var. durum) drought stress affect grain yield, which led to an increase in protein content by linking with better gluten strength and better bread-making quality in the drought environment, although other traits related to gluten quality and content as sodium dodecyl sulfate- (SDS)-sedimentation or mixograph mixing time were somewhat lower in that environment, which indicates probably qualitative changes at the protein level (Li *et al.* 2013; Magallanes-López *et al.* 2017). The analysis of the glutenins composition confirmed different effects of some alleles (Ma-

gallanes-López *et al.* 2017). Salt and drought stress in another work with durum wheat grown for two years on the field experiment caused the significant increase in grain protein content, wet and dry gluten contents, and SDS-sedimentation volume, whereby the impact of salinity stress was greater than drought stress (Houshmand *et al.* 2014). Flour protein content and SDS sedimentation volume increased, but not significantly, under drought stress conditions in durum wheat in the work of Li *et al.* (2013). In contrast, the gluten strength-related parameters such as lactic acid retention capacity and mixograph peak time increased significantly under drought. Drought also significantly enhanced flour yellowness (Li *et al.* 2013).

3. MAIN MECHANISMS OF A PLANT TO ADAPT TO DROUGHT CONDITIONS

The common drought-responsive mechanism comprises several characteristics: 1) Drought escape via completing plant life cycle before severe water stress conditions (e.g., early flowering) (Lisar *et al.* 2012; Abobatta 2019). 2) Drought avoidance via enhancing water taking capacity (e.g., developing root systems or conserving water by reducing transpiration such as closure/reduction of stomata and leaf area). 3) Drought tolerance via improving osmotic adjustment and increasing cell wall elasticity to maintain tissue turgidity. 4) Drought resistance via altering metabolic pathways under severe water stress condition. 5) Drought abandonment by reducing/removing a plant part (e.g., shedding mature leaves) (Prasad *et al.* 2017). 6) Drought-prone biochemical-physiological traits for plant evolution under long-term drought condition via genetic mutation and genetic modification (Xu *et al.* 2010). All these mechanisms may be involved consecutively or simultaneously in plant responses to drought stress (Xu *et al.* 2010; Han *et al.* 2015). Generally, C3 plants are better adapt to drought, because the response of C3 species to increased CO₂ is more positive than that of C4 species thanks to increased photosynthetic rate (Araus *et al.* 2002; Hamim 2005). Increased CO₂ increases the water use efficiency of C3 species because it causes a reduction in transpiration rate and an increase in CO₂ assimilation rate

of the plants (Hamim 2005). Studies have also suggested that C3 species may obtain more benefits of CO₂ enrichment under drought stress (Ward *et al.* 1999; Hamim 2005).

One of the most important molecules and best investigated in stress signalling is the plant hormone abscisic acid (ABA) (Davies *et al.* 2005; Daszkowska-Golec & Szarejko 2013; Takahashi *et al.* 2020). Endogenous ABA is rapidly produced in the plant during drought and triggers a cascade of physiological reactions, including stomatal closure (Davies *et al.* 2005) which is regulated by a signal transduction network. Stomatal activity, which is influenced by environmental stress factors, can affect the absorption of CO₂ and thus affect photosynthesis and plant growth (Osakabe *et al.* 2014).

Among proteins, heat-shock proteins (HSPs) (Di Donato & Geisler 2019) and dehydrins (Kosová *et al.* 2013) are involved in plant responses to a drought stress reaction. Chitinases (EC 3.2.1.14) and glucanases (EC 3.2.1.39) are other molecules activated in wheat by drought. Individual isoforms and their activity were rather stimulated under drought, especially in shoots (Gregorová *et al.* 2015).

3.1 Effect of ABA in cereals during drought conditions

Regulation of ABA under drought in plants is well discussed in many studies (Davies *et al.* 2005; Daszkowska-Golec & Szarejko 2013; Ghatak *et al.* 2017; Takahashi *et al.* 2020). However, the detailed molecular mechanisms of stress sensors and the regulators that initiate ABA biosynthesis in response to drought stress conditions are still unclear (Takahashi *et al.* 2020).

Plant roots reflect to the drying soil and produce signals, while ABA on transmission to shoots triggers stomatal closure to regulate crop water use through transpiration. However, transpiration is linked to crop growth and productivity, and limiting transpiration may reduce potential yield. While an early and high degree of stomatal closure affects photosynthesis (Osakabe *et al.* 2014) and hence biomass production, a late and low degree of stomatal closure exhausts available soil water rapidly which results in yield losses through a reduction in post-anthesis water use. Wheat genotypes differ in their ability to produce ABA under drought condi-

tions and also in their stomatal sensitivity to ABA. Root density distribution in the upper drying layers of the soil profile is identified as a candidate trait that can affect ABA accumulation and subsequent stomatal closure (Daszkowska-Golec & Szarejko 2013; Saradadevi *et al.* 2017). A simple collection of leaf samples to quantify ABA compared to extracting xylem tissue will facilitate rapid screening of a large number of germplasm for drought tolerance (Saradadevi *et al.* 2017).

An increased concentration of ABA in leaves associated with reduced stomatal conductance under water deficits has been confirmed in several studies conducted in various species including wheat (Saradadevi *et al.* 2017). A higher concentration of ABA was observed in wheat roots in association with increased root hydraulic conductance following excision of four out of five seminal roots. This increased concentration of ABA in root and subsequent enhancement of root hydraulic conductivity to meet increased transpiration demand is due to the redistribution of ABA from leaf to root (Kudoyarova *et al.* 2011). Thus, leaf ABA is involved in regulating root hydraulic conductivity, in addition to its role in regulating stomata (Saradadevi *et al.* 2017).

ABA has a central role in root-to-shoot drought stress signalling (Davies *et al.* 2005; Takahashi *et al.* 2020) and the regulation of functioning, growth, and development of plants in drying soil (Davies *et al.* 2005). Changes in xylem and apoplastic pH can affect the way in which ABA regulates stomatal behaviour and growth (Davies *et al.* 2005).

The lack of consensus among researchers in relation to a positive, negative, or neutral influence of ABA on grain yield suggests that the timing at which the water stress occurs is important. Pre-anthesis water stress, particularly during spike development and pollen meiosis, reduces grain number while post-anthesis water stress reduces grain size (Dolferus *et al.* 2011). This is because high ABA levels during the early reproductive stage affect grain composition and reduce grain number. On the other hand, during post-anthesis stages, high ABA levels promote grain filling by redistributing reserved carbohydrates to the grain (Liu *et al.* 2005). In one of the recent studies, when the soil water was exhausted rapidly after anthesis, the wheat cultivar Drysdale maintained a higher grain yield with a higher harvest index and

grain weight compared to the advanced drought-tolerant line IGW-3262. This was mainly because the cultivar Drysdale was more efficient at translocation of assimilates to grain (Saradadevi *et al.* 2015). Based on this study it appears that ABA negatively affects grain composition, but has a positive effect on grain filling by facilitating assimilate distribution to grain (Saradadevi *et al.* 2017).

3.2 The importance of HSPs in plant protection during drought

Heat shock proteins (HSPs) are a major component of multiple stress responses in plants (Guo *et al.* 2021; Kumar *et al.* 2020). HSPs are controlled by the action of diverse heat shock factors which are activated under stress conditions (Maaroufi & Tanguay 2013; Jacob *et al.* 2017).

By definition, protein denaturation is a constant direct or indirect consequence of any stress, as stresses are defined as factors limiting normal cellular functions carried out by proteins (Atkinson & Urwin 2012). Potentially, any stressor that induces protein misfolding would require HSPs. Thereby, chaperones are considered as powerful buffers against protein misfolding during environmental stress (including drought) and consequent genetic variations (Carey *et al.* 2006; Maaroufi & Tanguay 2013). The importance of HSPs is not limited to heat stress management, but these molecules are also involved in other stresses, such as cold, osmotic stress, drought, salt, UV, high light, oxidative stress, and pathogen infection (Swindell *et al.* 2007; Maaroufi & Tanguay 2013; Di Donato & Geisler 2019; Kumar *et al.* 2020; Guo *et al.* 2021).

The main inducers of chaperones are heat-shock factors (HSFs) and the diversity of the HSFs family in plants causes their study difficult. However, sequence and expression pattern comparisons showed both distinct and overlapping functions in stress resistance and development. The amount of free HSPs is the sensor of the cell capacity to maintain a stable proteome and feeds back on its own production. Indeed, in unstressed tissues, the commonly accepted “chaperone titration model” specifies that HSFs are sequestered by HSP70/90 and maybe other chaperones (Jacob *et al.* 2017). The HSP90 chaperone machinery controls multiple cellular processes by regulating the maturation, stability, activity, and

turnover of its substrates, the so-called client proteins (Di Donato & Geisler 2019).

119 DnaJ (HSP40) proteins (TaDnaJs; encoded by 313 genes) and 41 HSP70 proteins (TaHsp70s; encoded by 95 genes) have been identified and classified in wheat into six and four groups, respectively, via a phylogenetic analysis. An examination of protein sequence alignment revealed diversity in the TaDnaJ structural organization, but a highly conserved J-domain characterized by an HPD motif followed by DRD or DED motifs was observed (Guo *et al.* 2021). In the work of Kumar *et al.* (2020) using Position-Specific Scoring Matrix (PSSMs) and by sequence homology 753 *TaHSPs* including 169 *TaSHSP* (small HSP), 273 *TaHSP40*, 95 *TaHSP60*, 114 *TaHSP70*, 18 *TaHSP90*, and 84 *TaHSP100* were identified in the wheat genome. Compared with other grass species, the number of HSPs in wheat is relatively high probably due to the higher ploidy level and a large number of tandem duplication was identified in *TaHSPs*, especially *TaSHSPs*. The *TaHSPs* genes show random distribution on chromosomes, however, there are more *TaHSPs* in B and D sub-genomes as compared to the A sub-genome (Kumar *et al.* 2020).

Information about the involvement of HSPs in drought stress signalling is rare, however high expression of *TaSHSP* was observed during seed development, especially during the grain filling stage (Kumar *et al.* 2020). It was found that both the overexpression of HSC70 and the use of a dominant-negative form of HSP90 disrupt ABA-mediated stomata closure, thereby negatively affect water loss in stress conditions (Di Donato & Geisler 2019). The impact of ABA treatment on HSC70, HSP90, SGT1 (co-chaperone of HSP90, suppressor of G-two allele of *Skp1*), and RAR1 (co-chaperone of HSP90, required for *Mla12* powdery mildew resistance) was investigated by quantitative polymerase chain reaction (qPCR). A decrease in SGT1a mRNA and an increase in HSC70-4 mRNA was observed. HSC70-1 and HSC70-4 must therefore share the same function regarding the regulation of ABA signalling under physiological conditions. Only HSC70-4 is involved in mitigating ABA signals (Clement *et al.* 2011).

3.3 Dehydrins activation in cereals during drought

Dehydrins are significantly disordered proteins which are produced under water stress conditions. They play an essential role in the response of plants to abiotic stress and in adapting them to the stress conditions (Kosová *et al.* 2013; Thomas 2015). Their higher accumulation is usually induced in vegetative plant tissues under various stress factors that cause cell dehydration including salinity, lack of water, cold, and frost (Kosová *et al.* 2013). Several physiological studies oriented in plant response to stress have shown a positive relationship between the level of accumulation of transcripts or dehydrin proteins and plant tolerance to stress, especially in wheat and barley (*Hordeum vulgare* L.) (Kosová *et al.* 2013; Vítámvás *et al.* 2019).

The structural physicochemical and functional characterization of plant dehydrins and how their properties can be used to improve resistance to stress in plants has been described (Hanin *et al.* 2011). Dehydrins can non-specifically bind proteins and membranes contributing to the protection of their functions and structure from damage caused by environmental stresses. Dehydrins can also bind the DNA and act by not only protecting but also repairing the molecule under the attack of environmental stress factors (Liu *et al.* 2017).

Drought induces cell dehydration and expression of several dehydrins in wheat and barley. In durum wheat, the accumulation of YSK2 dehydrin DHN5 was studied in two cultivars exposed to drought stress. A different pattern of phosphorylation was observed in both studied cultivars, where the drought-tolerant cultivar reveals higher ranges of phosphorylation than the sensitive one (Kosová *et al.* 2014). Phosphorylation is associated with the different subcellular localization of the protein affecting the final function of the protein (Kosová *et al.* 2014). Quantitative and qualitative differences in low molecular weight dehydrin proteins were found in two barley cultivars with different drought tolerance capacity when plants were exposed to reduced field water capacity (Škodáček & Prášil 2011).

Qualitative differences in accumulated dehydrin proteins may be due to either the accumulation of different low molecular weight *Dhn* genes or allelic variants of the same genes, which differ in copy number of hydrophilic Φ segments and electropho-

retic mobility, as it was described for *Dhn4* (Kosová *et al.* 2014). The authors report that the gene expression is an indicator of drought stress and an appropriate adaptation mechanism and the demonstration of drought stress increases with the exposure time during the vegetative period and during the critical phases of plant development (Kosová *et al.* 2014; Klimešová *et al.* 2017).

3.4 Importance of antioxidants in plant protection against drought

In wheat, several studies have reported changes in the activity of many enzymes of the antioxidant defence system to control oxidative stress induced by environmental stresses. The enzymatic components comprise several antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, guaiacol peroxidase, peroxiredoxins, and enzymes of the ascorbate-glutathione cycle, such as ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase (Caverzan *et al.* 2016; Prasad *et al.* 2017; Jiang *et al.* 2019; Kapoor *et al.* 2020). Non-enzymatic components include the major cellular redox buffers ascorbate and glutathione as well as tocopherols, carotenoids, and phenolic compounds. Alterations in the activity of superoxide dismutase, ascorbate peroxidase, catalase, glutathione reductase, and guaiacol peroxidase and in the reactive oxygen species (ROS) concentration were reported in wheat plants in a field and at laboratory conditions (Caverzan *et al.* 2016; Cheng *et al.* 2016; Jiang *et al.* 2019). Furthermore, many reports demonstrate that the effect of abiotic stress in wheat is genotype-specific, where some genotypes show different responses in the same stress condition. Drought-tolerant genotypes generally maintained a higher antioxidant capacity resulting in lower oxidative damage (Devi *et al.* 2012; Caverzan *et al.* 2016). Wheat responses also depend on the tissue type, length, and intensity of the stress as well as on the developmental stage proving the complexity of the mechanisms of production and detoxification of ROS and the effect of ROS on antioxidant systems. Many studies have reported an increase in the concentration of H_2O_2 after exposure to a stress and its production depends on the intensity and duration of the stress factor (Alexieva *et al.* 2001; Caverzan

et al. 2016; Jiang *et al.* 2019).

Furthermore, the H_2O_2 level differs between various cellular compartments and is related to the type of stress (Caverzan *et al.* 2016; Dikilitas *et al.* 2020). The observed increase in enzymatic activities and decrease in oxidative damage are closely related. The expression of many antioxidant enzymes is positively correlated with higher tolerance levels against abiotic stresses. The activation of some enzymes leads to plant protection against oxidative damage. In rice plants, an important cereal model, increased concentration of antioxidant enzymes and increased expression levels of related genes have been connected with the plant response to drought (Caverzan *et al.* 2016).

Superoxide dismutases (SODs) are a family of key antioxidant enzymes that play a crucial role in plant growth and development and are regulated by development, tissue-type, and environmental signals (Jiang *et al.* 2019). In drought stress, SODs represent a frontline in the defence against ROS, they catalyse the dismutation of O_2^- (superoxide radical) to H_2O_2 (Gill *et al.* 2015). Catalases (CATs) are abundantly, but not exclusively, localized in peroxisomes and their function is to remove the H_2O_2 by reducing H_2O_2 to two molecules of H_2O . Drought-induced H_2O_2 accumulation is in a correlation with decreases in soil water content and CO_2 assimilation. Leaf H_2O_2 content increases even though total CAT activity doubling under severe drought conditions. Drought decreases abundance and modifies the pattern, of *CAT1* and *CAT2* mRNAs. The abundance of *CAT1* transcripts is regulated by circadian controls that persist in continuous darkness, while *CAT2* is modulated by light (Luna *et al.* 2005).

In wheat, a mutant line with reduced thylakoid ascorbate peroxidases (APXs) activity leads to regress in photosynthesis (Danna *et al.* 2003). Rice mutants double silenced for cytosolic APXs exhibit high guaiacol peroxidase activity, which can contribute to the cytosolic H_2O_2 scavenging in the vacuoles or apoplasts (Bonifacio *et al.* 2011). APXs catalyse the conversion of H_2O_2 into H_2O and use ascorbate as a specific electron donor. APXs proteins are distributed in chloroplasts, mitochondria, peroxisomes, and cytosol. The APXs genes show different modulation by several abiotic stresses in plants. The balance between SODs, CATs, and APXs is important

for determining the intracellular level of ROS, besides changes in the balance of these appear to induce compensatory mechanisms.

The expression of wheat glutathione peroxidase (GPX) genes was altered when wheat plants were exposed to salt, H_2O_2 , and ABA treatment. Moreover, other findings suggest that GPX not only acts as scavengers of H_2O_2 to control abiotic stress responses but also plays important roles in salt and ABA-signalling cascades (Zhai *et al.* 2013). In plants, the GPX proteins are distributed in mitochondria, chloroplasts, and the cytosol (Caverzan *et al.* 2016).

ROS are well recognized for playing a dual role, both as malignant as well as beneficial, depending on their concentration in the plant. The role of ROS as signalling molecules includes also processes related to growth, cell cycle, development, senescence, programmed cell death, stomatal conductance, and hormonal signalling (Inze *et al.* 2012; Caverzan *et al.* 2016). H_2O_2 is considered a signalling molecule in plants that mediates responses to various biotic and abiotic stresses. The biological effect of H_2O_2 is related to several factors, such as the site of production, the developmental stage of the plant, and its concentration (Wahid *et al.* 2007; Petrov & Breusegem 2012). Thus, due to the property that in low concentrations the H_2O_2 acts as a stress signal, many studies have demonstrated that its application can induce stress tolerance in plants (e.g., He *et al.* 2009; Caverzan *et al.* 2016; Jiang *et al.* 2019; Dikilitas *et al.* 2020). Low H_2O_2 treatments improve seed germination, seedling growth, and resistance to abiotic stresses (Caverzan *et al.* 2016). In wheat, it was observed that seed pre-treatment with H_2O_2 enhances the drought tolerance of seedlings (He *et al.* 2009). The exogenous H_2O_2 treatment also protects wheat seedlings from damage by salt stress and seeds pre-treatment enhances salt tolerance of wheat seedlings and decreases oxidative damage (Wahid *et al.* 2007). In rice plants, H_2O_2 not only acts as a toxic molecule, but also as a signalling molecule associated with salinity, cadmium, and ABA stresses (Kao 2014). In wheat, H_2O_2 plays an important role as a signal molecule, but also as a harmful chemical (Ge *et al.* 2013; Dikilitas *et al.* 2020).

Metabolic analyses at the level of phenolics showed an increase in the free and bound fraction

of phenolic acids almost exclusively in the shoots, and flavonoid isoorientin increased considerably as a protective action against oxidative stress (Gregorová *et al.* 2015).

4. BIOCHEMICAL RESPONSES TO DROUGHT IN CEREALS

During drought stress, plant cells accumulate soluble substances, contributing to a higher viscosity in the cytoplasm. The content of these special substances may become toxic under certain conditions and may cause problems in the formation of enzymes and the whole process of photosynthesis (Danna *et al.* 2003; Han *et al.* 2015). In a short-term drought, Rubisco is relatively stable and it decomposes just after a few days. Fixed-binding inhibitors may reduce the activity of the Rubisco within 24 hours. The rapid decrease in “dry” photosynthesis is accompanied by a reduction in the maximum rate of ribulose-1,5-carboxylate, the rate of regeneration of ribulose-1,5-bisphosphate, Rubisco, phosphoenolpyruvate carboxylase, NADP-malic enzyme, fructose-1,6-bisphosphatase, and orthophosphate-dikinase pyruvate (Reddy *et al.* 2004; Zhou *et al.* 2007). In addition, noncyclic electron transport is reduced to meet the requirements for reduced NADPH production, thereby reducing ATP and ROS synthesis (Reddy *et al.* 2004).

Different cultivars within crop species may strongly differ in their response and adaptation to drought stress (Abid *et al.* 2018). Studies at the transcriptomic level have revealed that the drought-tolerant and sensitive wheat genotypes can adopt different molecular strategies to overcome with drought stress. One of the main differences is the differential expression of some drought-inducible genes for cell protection (e.g., antioxidants, detoxifiers, dehydrins, transporters, and compatible solutes), regulation (e.g., kinases, transcription factors, and hormones), and remodelling of cellular components (e.g., membrane systems, cell wall, and primary metabolic networks). A large number of these genes are often activated in drought-sensitive wheat genotype, while a tolerant genotype shows a constitutive expression of several genes activated in a sensitive genotype and that contribute to limiting drought effects and

perception. In addition, signal transduction and hormone-dependent regulation pathways are also different in various wheat genotypes. The drought-tolerant genotype can quickly sense drought and trigger the signal transduction pathways for activation of downstream elements for survival from drought stress (Cheng *et al.* 2016).

5. MOLECULAR TOOLS FOR IMPROVEMENT OF DROUGHT TOLERANCE IN CEREALS

Drought tolerance is not a qualitative trait, but a complex of quantitative plant traits controlled by numerous genes and other plant traits with minor individual effects (Senapati *et al.* 2018). In recent years, knowledge about molecular regulation has been generated to understand drought stress responses. For example, information obtained by transcriptome analysis has enhanced our knowledge and facilitated the identification of candidate genes that can be utilized for plant breeding. On the other hand, it becomes more evident that the translational and post-translational machinery plays a major role in stress adaptation, especially for immediate molecular processes during stress adaptation. Therefore, it is essential to measure protein levels and post-translational protein modifications to reveal information about stress-inducible signal perception and transduction, translational activity, and induced protein levels (Ghatak *et al.* 2017). Research in transcriptomics, proteomics, and metabolomics is increased to understand the mechanisms of drought tolerance in plants (Swindell *et al.* 2007).

Proteomics has become the most direct and powerful tool to obtain protein expression information of plants responses to drought stress, thereby complementing transcriptomic studies. Comparative proteomics of drought-tolerant and sensitive wheat genotypes is a strategy to understand the complexity of molecular mechanism in wheat in response to drought stress (Cheng *et al.* 2016). Dehydrins have been reported as important factors in stress tolerance and the genetic locations of some dehydrin family members co-locate with QTL for drought tolerance in barley (Thomas 2015). Of the antioxidant components, *TaSOD* genes are involved in the regulation

of wheat tissue development and likely play important role in response to abiotic stress including a longer duration of drought. Four of these genes (*TaSOD1.1a*, *TaSOD1.4*, *TaSOD2.1*, and *TaSOD2.3*) showed up-regulation in leaf and five genes (*TaSOD1.7*, *TaSOD1.9*, *TaSOD1.11a*, *TaSOD2.1*, and *TaSOD2.3*) showed up-regulation in the root (Jiang *et al.* 2019).

Efforts to improve crops tolerance to drought and associated soil salinity are considerable, especially in the context of climatic change and irrigation water scarcity. Using the combination of DNA fingerprints of different genotypes with phenotypic measurements, specific chromosomal regions – so-called quantitative trait loci (QTL) – were associated with the expressed traits. Specific DNA markers have been linked with favourable QTLs using the technology of marker-assisted selection (MAS) (Tuberosa & Salvi 2006). Thanks to the advances in next-generation sequencing, the identification of major QTLs regulating specific drought responses is successful and via the development of large numbers of genetic markers such as single nucleotide polymorphisms (SNPs) and insertion-deletions (InDels) it represents an efficient way to improve drought tolerance in cereal crops (Kole *et al.* 2015). Studies reporting major drought-responsive genes and QTLs in wheat have recently been published (Choudhary *et al.* 2019; Khadka *et al.* 2020; Pang *et al.* 2020) suggesting that for the past decade, QTL has been the focal target of research to identify the genetic loci regulating the adaptive response of crops to drought stress (Zenda *et al.* 2020).

In addition to the classical and molecular plant breeding techniques, the key tool became the transfer of genes and gene regulatory regions related to plant water management. Transgenic approaches using candidate genes such as transcription factors, microRNAs, as well as genome editing technology have been well summarized (Tuberosa & Salvi 2006; Abdallah *et al.* 2015; Paul & Roychoudhury 2018; Jha 2019; Anwar & Kim 2020). Attractive targets of plant genetic engineering for drought tolerance are considered transcription factors (e.g. WRKY, MYB, NAC, AP2/ERF, GBF3). Their potential in the engineering of stress-tolerant monocot plants has been already reviewed (e.g., Javed *et al.* 2020). The subjects of interest are also signal molecules

and genes encoding them to help the plants to cope with abiotic stresses (Ahmed *et al.* 2020). Many genes participating in drought stress response have been already identified and transferred into cultured plants, including the most important cereals. Transgenic wheat, barley, maize, rice, and other cereals with increased water use efficiency and drought tolerance were experimentally developed (Gao *et al.* 2005; Napolean *et al.* 2018; Zhou *et al.* 2018; González *et al.* 2019; Khan *et al.* 2019; Oladosu *et al.* 2019). However, an only a very limited number of drought-tolerant cereal cultivars created by genetic transformation are so far registered for commercial use. The most important databases of approved genetically modified plants managed by the International Service for the Acquisition of Agri-Biotech Applications (ISAAA's GM Approval Database, <http://www.isaaa.org/gmapprovaldatabase/>) and the European Community register of GM food and feed (https://ec.europa.eu/food/plant/gmo/eu_register_en) include at present time only drought tolerant genetically modified maize, sugarcane, and soybean. Drought tolerance of maize was obtained by the introduction of the gene *cspB* that encodes the cold shock protein B. The *cspB* transgene maintains normal cellular functions under water stress conditions in the plant by preserving RNA stability and translation.

Another co-solution of abiotic stress problem in plants is a better understanding of interactions between plants and plant growth-promoting microorganisms (PGPM). They can protect plants from the negative effects of drought and salinity (Khan *et al.* 2020; Ma *et al.* 2020). Biotechnology and gene transfer technologies could increase the effectiveness of plant-microorganism interactions. Genetically engineered PGPM enhance the survival of plants under water deficiency as it has already been proven in plants colonized by genetic engineered soil bacteria over-producing trehalose (Vilchez *et al.* 2016).

Biotechnology tools and genetic engineering approaches, along with a better understanding of plants, and other stress mitigation strategies, are solutions for the very near future crop production with limited water resources.

6. CONCLUSIONS

Plant responses to stress are dynamic processes, which are able to enhance tolerance/resistance mechanisms and establish metabolic homeostasis under extreme environmental conditions. Regulatory proteins play an important role in regulating the alterations under water deficit conditions and represent some of the most important targets for crop improvement. Protein phosphorylation plays an important role in signal perception and transduction under drought stress. Several kinases and phosphatases, and other signalling proteins are regulated leading to stress adaptation and possible tolerance mechanisms like stomata closure. Stress acclimatization is an energy-consuming process which is indicated by alterations in energy metabolism. Therefore, increased abundance of the enzymes involved in glycolysis is during drought observed. Drought stress leads to an enhanced risk of protein damage due to cellular instability/imbalance. Therefore, several proteins with protective mechanisms show increased levels (for example HSPs, namely HSP70, HSP90). Disturbed cellular metabolism leads to oxidative damage and hence increase in ROS scavenging enzymes was also observed (SOD, GST, CAT, APX, and others) which is one of the common and practical features in plants under drought stress.

Sustainable crop production is the major challenge in the current global climate change scenario. Drought stress is one of the most critical abiotic factors which negatively impact crop productivity. Cereals are the major food crops that are necessary for the survival of the entire human population across the globe. Not only humans but also livestock farming depend on these crops. Considering all the drastic global climatic changes, our food security and its distribution is a big question. Marker-Assisted Breeding and SMART Breeding Techniques are key in agricultural productivity. Proteomics is one such tool to improve SMART breeding. The integration of genomics, proteomics, transcriptomics, and metabolomics is important for future research in green systems biology to improve its application in cereal crops. Increasing the resistance of wheat to drought and drought-related stresses is one of the most important objectives of breeding programs. However, from the point of view of current use in plant pro-

duction, the greatest advances in the development of plants tolerant of drought and other abiotic stresses have been made through genetic engineering technologies.

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RAPID IDENTIFICATION OF RICE MACRONUTRIENT CONTENT IN SALINE SOILS USING SMARTPHONE CAMERA

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Indonesia's rice production has decreased by 6.83% (on average) in the last five years (2015–2019) because of some factors. Salinity (42%) is one of the leading factors that cause decreasing rice production besides climate change (21%), drought (9%), and other factors (28%). The smartphone camera serves as an alternative technology to prevent macronutrient deficiencies due to salinity. This study used aerial photos from android with visible light (R, G, and B), and the image was taken from a height of 5 m. The observation of macronutrient content in plant biomass was carried out using a free grid to adjust rice fields and saline soil. The formula was obtained from regression analysis and paired t-test between the biomass macronutrient and the extracted digital number of aerial photographs that have been stacked. The results showed that digital number (DN) from a smartphone was reliable to predict nitrogen (N), phosphorus (P), and potassium (K) content in rice with formula $N = 0.0035 * DN + 0.8192$ (R^2 0.84), $P = 0.0049 * DN - 0.2042$ (R^2 0.70), and $K = 0.0478 * DN - 2.6717$ (R^2 0.70). There was no difference between the macronutrient estimation results from the formula and the field's original data.

Key words: remote sensing, visible light, android, nitrogen, phosphorus, potassium, salinity

Rice is one of the world's vital food commodities in which productivity is predicted to decline in 2020 by 0.60% (USDA 2020). Based on the (World Agricultural Production.com, 2020), Indonesia ranked third in rice producers, with production reaching 36.5 million metric tons. In Indonesia, rice is an essential food commodity and is the primary foodstuff for the community. The increase in rice demand is not matched by increased production, which fell by –6.83% (on average) from 2015 to 2019 (Central Bureau of Statistics 2020).

Compared to other problems that cause fluctuations in rice production, salinity still has a more severe impact – another case related to water availability. A study by (Iswari *et al.* 2016) mentioned that rice production is caused by drought at the re-

search location, namely the Demak Regency, which resulted in crop failure of 0.629% in 2013, 8.121% in 2014, and 9.173% in 2015. Another problem that has a similar effect to salinity is climate change. Rice production has decreased due to climate change in Indramayu Regency (Ruminta 2016), with an average decline of 21% to 40%. Rice is often affected by salinity, reducing 42% production (Ahmed & Haider 2014). Rice can adapt to almost any environment from lowland to highland. In Indonesia, rice cultivation is carried out in various lands, including wetlands in lowland rice fields, dry land, upland rice fields, and peatlands (Utama 2015). A study by Mardiansyah *et al.* (2018) stated that the Cihorang variety has moderate salinity tolerant characters. The Inpari 32 variety is an inbred from the selec-

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tion results of the Ciherang variety, and Inpari 42 is a salinity tolerant variety (Agricultural Research and Development Agency, 2019).

Soil salinity shows the concentration of dissolved salt in the soil (Sembiring & Gani 2010). Salinity occurs due to (1) the high intake of water containing salt, for example, due to seawater intrusion, (2) higher evaporation and evapotranspiration than precipitation (rainfall), and (3) soil parent material containing salt deposits (Rachman *et al.* 2018). Lowland rice production due to salinity stress can cause a decrease in production. The effect of salinity on crops includes osmotic pressure, nutrient balance, and NaCl⁻ salts' toxic impact on saline soils that can disrupt the nutrient balance because certain nutrients are excess or reduced. Potassium is exchangeable, which means a decrease in these elements' availability affects other nutrients crops (Setiawan & Herdianto 2018). The salinity symptoms in rice crops begin with dry leaf tips, reduced tillers, root length, crop height, shoot dry weight, and root weight. Salinity suppresses crop growth processes with effects that inhibit cell enlargement and division, protein production, and the addition of crop biomass. Crops that experience salt stress do not respond directly to damage, but growth is depressed and changes slowly. Excess Na⁺ in crop cells directly damages membrane systems and organelles, causing abnormal growth and development before crop death (Sayed & Sayed 2013).

The use of smartphones to identify the macronutrient content of biomass is interesting to study. This technology has potential because many people have used smartphones, have high resolutions, and can be used quickly. One of the most widely used smartphone platforms is an android (>80% smartphone user use). Android is a Linux-based operating system for smartphones that includes an operating system, middleware, and applications (Safaat 2011). Some android cameras' sensors are ambient light sensors, temperature and humidity sensors (Maulana & Setiawan 2018).

In the previous research, Setiawan and Herdianto (2018) created a mobile application that could analyse and recommend the need for nitrogen in rice plants based on the rice leaves' colour. In this application, a set of process stages for image processing and classification is implanted. It is used to analyse

the colour of rice leaves captured via an android camera. Image processing is a feature extraction of red, green, and blue (RGB) values to obtain features in leaf colour images. From the accuracy testing results, the application accuracy rate in analyzing and recommending nitrogen needs on average is 66.67%.

This study aims to implement an android camera to monitor macronutrient content in saline fields. The salinity level in a plot/landscape is considered a common problem. The previous study conducted smartphone camera use (Astika *et al.* 2011), macronutrient analysis, and salinity analysis (Grattan & Grieve 1998). It is necessary to have a technological breakthrough that can "photograph" the variability macronutrients in the salinity area. The breakthrough is the use of terrestrial cameras. The implementation of agricultural precision is planned for modern technology by utilizing industrial era 4.0 technologies such as terrestrial cameras. Therefore, this study measures how accurate terrestrial cameras are in analyzing salinity problems in rice crops. Hopefully, this study can support the government's food security program (NAWACITA) and SDGs.

MATERIAL AND METHODS

Research location

The research activity was carried out in the rice fields in the Jabon district, Sidoarjo Regency, and East Java (Figure 1). Jabon district is located in a lowland, with coordinates of 112° 70' 36.17" – 112° 87' 33.13" East longitude and 7° 49' 40.01" – 7° 57' 83.45" South latitude. The study area has an annual rainfall ranging from 1,300–1,700 mm per year, with the number of rainy days ranging from 80–120 rainy days per year. The average air temperature per year in this area ranges from 21–34°C with a relative humidity level of ± 76% (Climate-Data.org). There are three reasons why choosing a small place like Sidoarjo Regency as the research location, namely 1) It is easier to generate basic data for the algorithm; 2) The large image capacity of the smartphone camera becomes inefficient if applied to a large area, and 3) Unique landforms. The Jabon Subdistrict area is formed from the river and sea sedimentation or fluvio-marine.

One of the characteristics of this landform is high salinity because the material contains salt deposits. Many people take advantage of it by cultivating rice, although the harvest in recent years has decreased. The same landform character is found in Central Java, Rembang Regency. It is also formed from the sedimentation of rivers and seas. Erosion material is deposited by rivers on the coast and combined with material carried by ocean waves (Wulan *et al.* 2016).

Jabon district is located in a coastal lowland area, which topography condition is influenced by fluvio-marine sediment and alluvium material (Marsoedi *et al.* 1997). The soil types in the Jabon district include Typic Hydraquents (Soil Survey Staff 2014). The rice field is an area of 1,883.86 ha, or 23.05% (from the total area). Jabon district consists of two alluvial and marine landscapes. The alluvial landform with the alluvial plain sub-landform is in the western part of 2,417.01 ha or 29.6%. Marine landform with plains tidal sub-land is east of Java Island, covering 5,756.34 ha or 70.4% (Marsoedi *et al.* 1997).

Material

A smartphone camera took the image in RGB format with 48 megapixels. This terrestrial camera

had 4GB RAM and a 4,000 mAh battery. The image had a size of 4,000 × 3,000 pixels. Another tool used was a 5 m long pole as a vertical photo-taking tool. Then there was the Gimbal for the terrestrial camera stabilizer (Astika *et al.* 2011).

Field observation

The research location was determined based on the initial salinity analysis with an Electrical Conductivity value of 9.6 mS/cm and exchangeable Na of 1.8 cmol/kg. This study was located in two transects. The length of Transect 1 was ± 4 km, and Transect 2 was ± 3.4 km. Transect 1 was 316–817 meters, and Transect 2 was 104–718 meters, where each transect had ten observation points. The salinity source’s distance was 10.65 km – point determination based on a free grid (Raves 2007). Transect 1 consisted of observation points 1a to 10a, which had a distance of 11.2 to 13.4 km from the salinity source. Additionally, transect 2 consisted of observation points 1b to 10b, which had a 10.3 to 13.5 km distance from the salinity source.

The point determination could be seen from soil analysis results (saline indication) and the coast distance. The rice and soil samples were taken using an

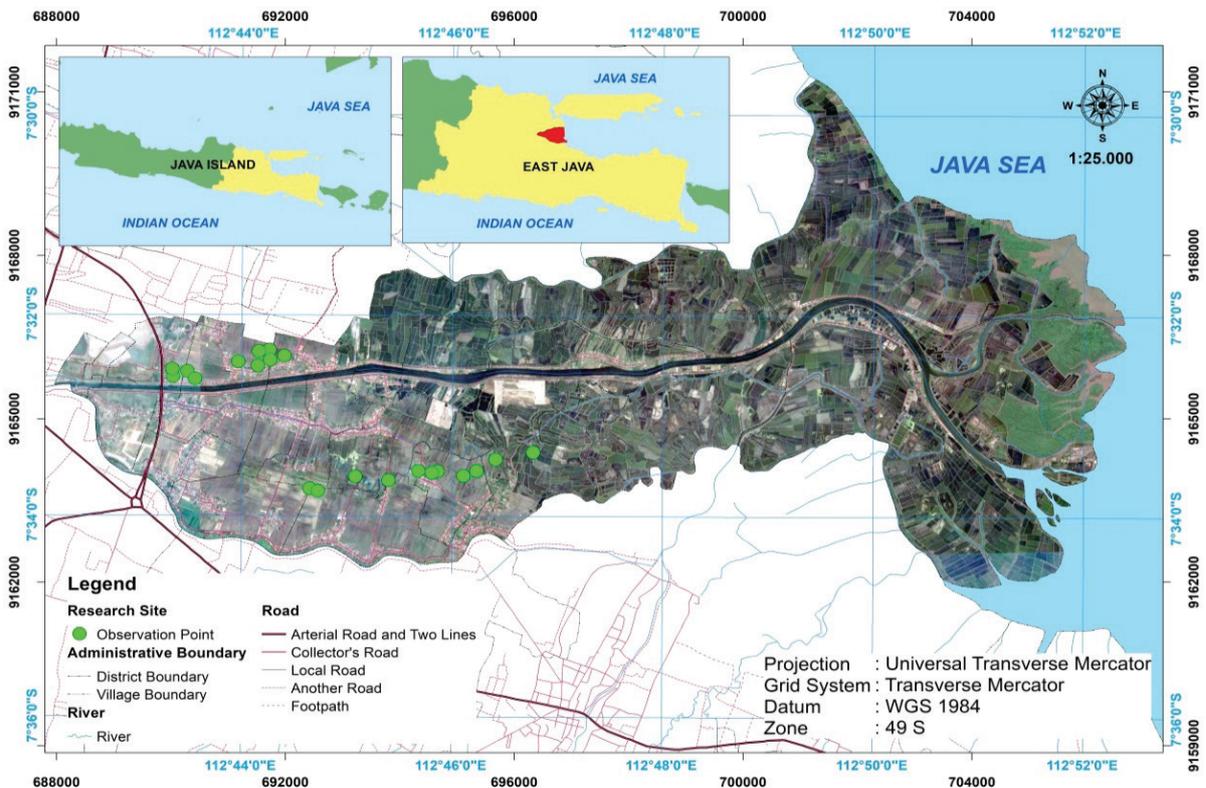


Figure 1. Distribution of research observation points

active field survey method by constructing transects through the ArcGIS 10.3 application. Determination of the sampling point is taken based on field conditions / without a rigid grid and directly tagged to take the location's coordinates. Determination of observation points is done by purposive randomized sampling (Raya 2007). The soil and rice sampling methods are described in "Soil and crops sampling" section (page 65). The research location was determined based on the age of the crops (in the vegetative phase). The age of rice crops obtained in the survey activity was between the ages of 41 to 56 DAP (days after planting). The plants were classified into the vegetative phase of class 2, rice crops aged 41–64 DAP from the age data. Detection of rice crops in rice fields with a good image is in the vegetative phase 2 (LAPAN 2015). This stage also involved the preparation of tools and materials. Then the tools needed for the field survey were prepared, such as a trowel, SPAD (chlorophyll meter), dreadlocks, 5 meter long sticks, administrative maps of Jabon Regency, and other supporting maps (Raya 2007).

Photo-taking using smartphone camera

The tool used was a smartphone camera with 48 megapixels and visible light wavelengths (red,

green, and blue). A gimbal smartphone camera supported the camera to stabilize, and the images were taken from a height of 5 m. The photoshoot was carried out in the rice field with an area, and the land adapted to the existing land with varying levels of leaf colour (Astika *et al.* 2011). The gimbal stabilizer can stabilize the movement and disturbance of the wind because the actuator system in the gimbal design uses a *servo motor* (Fahmizal *et al.* 2018). Servo motor is an electrical device used in machines that function to push or rotate objects with high precision control in angle, acceleration, and speed. The angle of elevation of the gimbal will be controlled stably (Suryana 2018).

The first step in taking photos using a smartphone, apart from making sure the camera is functioning correctly, is paying attention to suitable weather conditions. Overcast skies or the hot sun will affect the image. Smartphone photos require side lap, overlap, and image height settings so that errors that occur due to movements such as tilt and poor lighting can be avoided (Syauqani *et al.* 2017). The difference in the tilt and angle of sunlight, shooting is carried out simultaneously at 10:00 a.m. and a minimum height of 5 meters for smartphone camera use. Research conducted by Simanungkalit

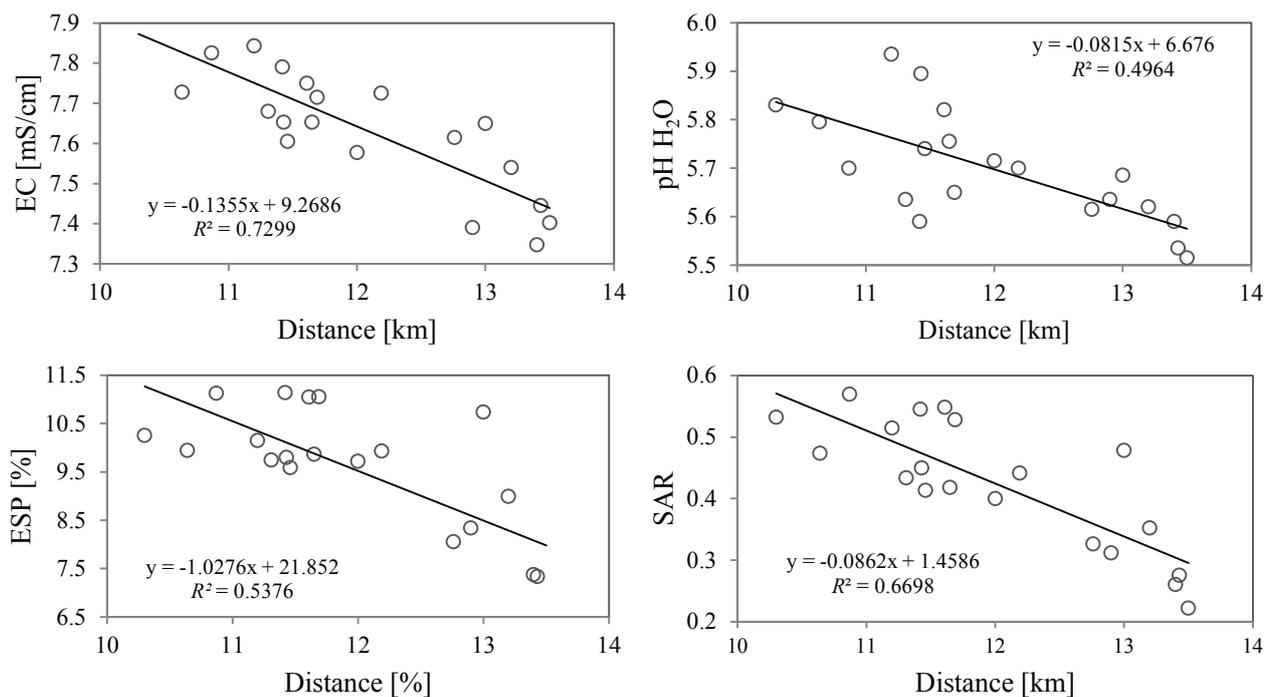


Figure 2. Graph of soil salinity indicator. EC –Electrical Conductivity; ESP – Exchangeable Sodium Percentage; SAR – Sodium Adsorption Ratio; pH H₂O based on the distance from the salinity source

et al. (2019) found that smartphone photos obtained from images taken at 10:00 a.m. had an aerial photo accuracy rate of above 95% in the perfect category.

Soil and crops sampling

The survey activities were carried out by taking soil and crop samples. The soil and crop (leaf) samples were taken before or after aerial photographs on the same day. This step was taken so that the soil samples taken did not undergo significant changes in the field conditions. Real-time sampling was also carried out so that the resulting data can have high accuracy values. The soil sampling taken was a layer of the rice root area. The soil sampling was done on topsoil using the undisturbed soil sampling method (Putra & Nita 2020). The soil sampling was taken from a depth of 0–0.2 m (± 1 kg) (Vadas *et al.* 2006). The sampling of rice biomass was carried out by taking part in the leaves. The determination of soil and crop sampling points based on field conditions/without rigid grids and directly tagged to retrieve location coordinates using GPS. The decision of the observation points was carried out by purposive randomized sampling (Rayaes 2007). The composite soil sampling and rice leaf samples were conducted for laboratory analysis to obtain levels

of nitrogen (Bremner 1996), phosphorus (Bray & Kurtz 1945) and potassium (Zakiyah *et al.* 2018) as macronutrients.

Salinity parameter analysis

In identifying macronutrients in rice crops in saline soils, salinity parameters must be considered. Salinity analysis was evaluated using the percentage of sodium exchange (ESP), soil pH, electrical conductivity (EC), and sodium adsorption ratio (SAR) (Djuwansah 2013). This parameter is called the salinity indicator. Salinity parameter criteria are exchangeable sodium percentage (ESP) < 15% (Gupta & Sharma 1990), soil pH < 8.5 (Amran *et al.* 2015), sodium adsorption ratio (SAR) < 13 (Robbins 1984), and electrical conductivity (EC) is 2–4 or > 4 mS/cm (Rhoades *et al.* 1989) in soil. Saline soil is different from saline-sodic and sodic soil. Saline-sodic has criteria of EC > 4 mS/cm, ESP > 15%, and pH > 8.5. Sodic soil has criteria of EC < 4 mS/cm, ESP > 15%, and pH > 8.5 (Sipayung 2003).

Image pre-processing and digital number extraction

The pre-processing of smartphone aerial photos was an initial data processing process. There were several pre-processing stages performed (Muñoz & Kravchenko 2011). The first stage was rectifica-

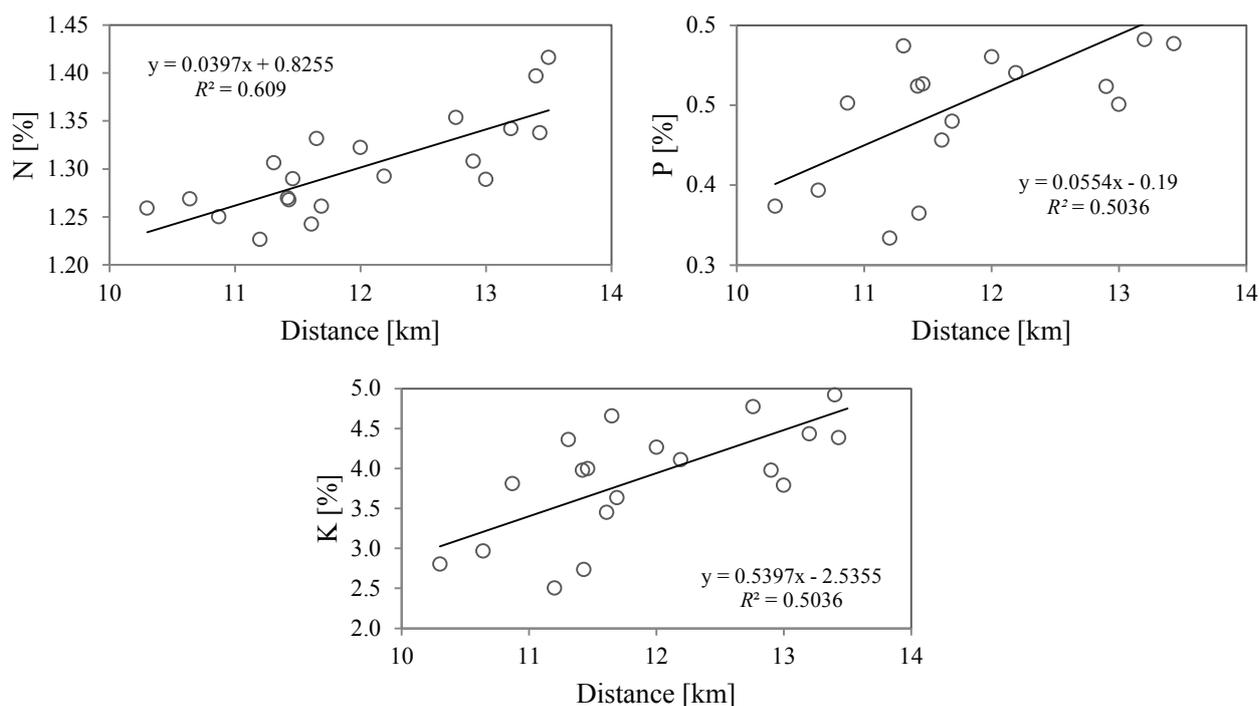


Figure 3. Plant biomass analysis result and the distribution based on distance from saline source
Note: N – nitrogen; P – phosphorus; K – kalium

tion. The second step is to extract digital numbers in ArcGIS 10.3 using the Extract Value to Points Tool (Putra & Nita 2020). This tool is used to obtain the digital number of each pixel at the raster observation point (Dell 2009). Digital numbers are RGB values because rasters are made up of red, green, and blue waves. In this study, the digital number value is the total value of the red, green, and blue waves in one pixel (RGB combination). The digital number transformation results were continued with statistical analysis of correlation and regression using *R* software and then compared with laboratory data correlated with rice crops.

The point of taking the digital number reclaimed aerial photo made diagonally. In one aerial photo, there were five digital number value retrieval points. Each DN value retrieval point was three replications (15 points in total), then averaged into one aerial photo's value (Astika *et al.* 2011). The smartphone camera aerial photos were entered into the ArcGIS 10.3 application. The rectification was carried out with the coordinates of the rectification coordinates in each aerial photograph corner. It was then cor-

rected, and the digital number extraction was started by adding an improved aerial photo.

Moreover, the digital number extraction was carried out. The extraction of digital numbers on a smartphone camera was carried out using each rectified photo, and then a sampling point was entered. This study was conducted using visible (red, green, blue) channels obtained through shooting using smartphone cameras.

Statistical analysis

Initially, the laboratory data results and the normality test result for potato production were analyzed using R studio by the Shapiro-Wilk method (Royston 1992). The correlation test was used to determine the closeness of the relationship between variables and the direction of the relationship (Putra & Nita 2020) The correlation coefficient value (*r*) was compared to the *r*-table (Bewick *et al.* 2003).

Formulation and interpolation of macronutrients deficiency in saline soil

The equation was used to estimate macronutrients (nitrogen, phosphorus, and potassium) of rice crops in salinity stress. Moreover, the resulting equations were associated using an aerial photo with a raster calculator on ArcMap 10.3 in ArcToolbox on the Map Algebra tool (Lubis 2011). The resulting interpolation estimated macronutrients (nitrogen, phosphorus, and potassium) in rice biomass.

Accuracy assessment

The validation test used a paired t-test to verify the correctness or certainty of a model. The reliability test using R studio aimed to determine whether model consistency accuracy – validation using the T-pair test (Montolalu & Langi 2018).

RESULTS

Soil salinity analysis results

Based on Figure 2, the values of electrical conductivity (EC), SAR, ESP, and pH H₂O are getting bigger and closer to the source of salinity. The image is included in the classification of moderate salinity values. The electrical conductivity obtained in this study ranged from 7.35 to 7.92 mS/cm. According to Sipayung (2003) classification based on electrical conductivity values, moderate salinity is rated

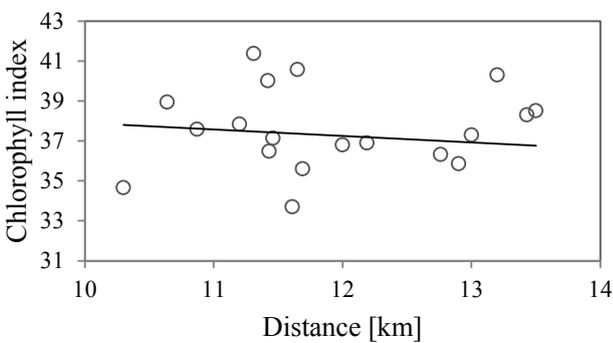


Figure 4. Graph of chlorophyll index

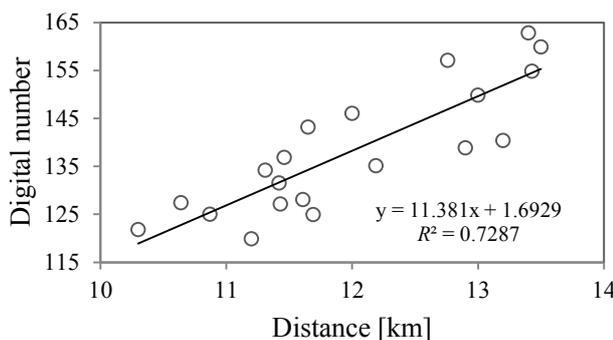


Figure 5. Digital number distribution based on the distance of the saline source

at 4–8 mS/cm, where many plants are affected. It includes control in SAR because the value is < 13, ESP includes control because all points have ESP values < 15%, and pH < 8.5. That value corresponds to the saline soil.

The chemical properties used as criteria for saline soils are characterized by soil EC more than 4/ > 4 mS/cm, EC (Electrical Conductivity) < 15%, and pH < 8.5 (Pardo 2010). The salinity indicator consisting of EC, pH H₂O, ESP, and SAR showed an increase in the value of each indicator as the distance from the saline source got closer (Figure 2). The EC values at all observation points increased to 7.4 mS/cm at a distance of 13.5 km, then increased to about 7.9 mS/cm at a distance of 10.5 km. The same pattern of increase also occurred in pH H₂O and SAR. Soil H₂O pH increases from about 5.5 to almost 6 as the salt sources are closer to each other. Another indicator is that the SAR increased from 0.2% to 0.6%, and the ESP increased from 6 to around 10. Therefore, the closer to the saline source, the higher the salinity level. This condition causes the area near the source of salinity to become an area of high salinity flow to crops.

Macronutrient biomass analysis results

Analysis of nitrogen, phosphorus, and potassium biomass:

The laboratory analysis of nitrogen, phosphorus, and potassium crops (leaves) showed that the total nitrogen, phosphorus, and potassium levels of rice crops decreased towards the saline source at the observation point. The results of the study of the total nitrogen, phosphorus, and potassium values of rice crops showed a decrease as the distance began to get closer to the source of salinity (Figure 3). Salinity is very influential, where the higher the salinity value causes inhibition of nutrient absorption for crops. Salinity interferes with crops' nutrient uptake in two ways. First, the ionic strength of the substrate, regardless of its composition, can influence nutrient uptake and translocation. Second, salinity interferes with plant mineral relationships by reducing nutrient availability through competition with ions (Monica *et al.* 2014).

Leaf chlorophyll index

Chlorophyll is one of the factors to determine the

status of nutrients in leaves. Figure 3 has the lowest chlorophyll value of 30.8 units/SPAD, and the highest is 41.38 units/SPAD. According to Prabowo *et al.* (2018), chlorophyll measurement results with SPAD values can be categorized into three criteria, namely low (< 50), medium (50–53), and high (> 53). The chlorophyll in this study had a SPAD value of < 50 came in the low category. The difference in rice chlorophyll in various varieties was due to crops' ability to adapt to different salinity conditions.

As shown in the graph in Figure 4, the chlorophyll's value does not increase or decrease significantly. The salinity source's far and proximity do not affect the increase or decrease in chlorophyll's value. This condition is due to the difference in the age of crops and rice varieties used.

The average age of crops at a distance of 13.4 km to 11.2 km from salinity source was 50 DAP (the day after planting), with the youngest crops age 42 DAP. Besides, in on-point observation, with a distance of 13.5 km to 10.3 km from the salinity source, the average age of crops was 54 DAP with the oldest age of 58 days after planting and the youngest of 50 DAP. The varieties used at all observation points were Ciherang, Inpari 32, and Inpari 42 varieties. The chlorophyll index was reviewed from varieties. The average value of chlorophyll in the Ciherang variety was 36.1 units, Inpari 32 variety was 37.6 units, and Inpari 42 was 38 units. According to (Banyo *et al.* 2013), in terms of crop life, crops with a more extended planting period cause a higher chlorophyll concentration than plants that grow faster in the vegetative phase. According to (Muyassir 2012), crop age affects the value of chlorophyll in leaves. Mardiansyah *et al.* (2018) stated that the Ciherang variety has moderate tolerant characters to high salinity. Inpari 32 is an inbred variety from the Ciherang selection, and Inpari 42 is a salinity tolerant variety (Suhartini & Zulchi 2018). Their results stated that salinity did not affect the crop's chlorophyll levels (Nurgayah & Irawati 2017).

Digital number extraction

In this study, the digital number (DN) used is a combination of RGB. Extraction of digital numeric values is taken from the total values of the red, green, and blue pixels visible in the aerial pho-

to. Pixels (picture elements) are the minor element points in a photo. The numeric number (1 byte) of a pixel is called the digital number (DN) (Efendi 2012). The use of smartphones can be implemented independently (stand-alone) by storing data on the mobile device (for simple applications) (Gunita *et al.* 2013). The smartphone's camera uses visible RGB (red, blue, and green) electromagnetic waves. Unlike terrestrial cameras, UAVs, drones, and others, the DN value uses visible light (RGB), NIR or SWIR, or Red Edge. However, if used a vegetation/soil index, the name is an index number, not a digital number (Bernardi *et al.* 2017). The use of smartphones is developing satellite imagery and UAV research with a higher level of precision. Salinity characteristics between locations are different, so it is necessary to use smartphones (Astika *et al.* 2011).

The digital number extraction is started with adding RGB photos. Then a digital number extraction is performed. Digital number extraction on a smartphone camera is done using each photo rectified and then inserted sampling point. In one aerial photo, there are five digital number value retrieval points. Each DN value retrieval point consists of three replays (15 points in total), then averaged to one aerial photo's value. The data of digital number extraction results through aerial photos utilizing smartphone camera obtain results with details based on Figure 5.

The highest digital number value is obtained at 162.8, while the lowest at 119.4. The highest digital number value is obtained at 159.9, while the lowest at 121.8. The digital number's value increases to point ten, the digital number value decreases. The closer the salinity of the source, the digital number values decrease (Figure 5).

Statistical analysis result

Normality test:

The observation variable carried out by the normality test can be said to be normal if the p-value is $p \geq 0.05$. The Digital Number smartphone camera has a normality test value of 0.236. N total [%] crops have a normality test value of 0.340. Then, from the availability of nutrients in the biomass, namely nitrogen total [%], crops have a normality test value of 0.340, phosphorus total [%] value of 0.601, and potassium total [%] value of 0.604. From the availability of nutrients in the soil, namely nitrogen total [%], the soil has a normality test value of 0.246, P₂O₅ total [mg/100g] value of 0.614 and K₂O total [mg/100g] value of 0.574. EC [mS/cm] has a normality test value of 0.627. Salinity indicators can be seen from pH H₂O data has a normality test value of 0.734; pH KCl data has a normality test value of 0.530, ESP value of 0.254, and SAR value of 0.298. All parameters data can be said to

T a b l e 1

Correlation analysis of parameters with smartphone camera digital number

	DN	Chlorophyll	EC	pH H ₂ O	ESP	SAR	N [%]	P [%]	K [%]
DN	1	-0.11	-0.81	-0.70	-0.78	-0.84	0.91	0.84	0.84
Chlorophyll		1	0.15	-0.12	0.10	0.06	-0.02	0.11	0.11
EC			1	0.63	0.83	0.92	-0.83	-0.69	-0.69
pH H ₂ O				1	0.55	0.60	-0.71	-0.84	-0.84
ESP					1	0.97	-0.87	-0.62	-0.62
SAR						1	-0.91	-0.71	-0.71
N [%]							1	0.87	0.87
P [%]								1	1.00
K [%]									1

Description: EC – Electrical Conductivity; ESP – Exchangeable Sodium Percentage; SAR – Sodium Adsorption Ratio; pH – acidity. Note: N – nitrogen; P – phosphorus; K – kalium

be expected because the value is more than 0.05. All parameters can be said to be expected so that they can be continued to the correlation.

Correlation between salinity and biomass nutrient availability

The correlation test between parameters and smartphone camera digital number values is presented in Table 1. The macronutrients (nitrogen, phosphorus, and potassium) and salinity indicators in rice crops based on the smartphone camera digital numbers are processed from smartphone regression test equations camera digital number values and the results of the analysis of crops and soil samples in rice crops in the field.

The equation used is the equations of smartphone camera digital numbers. Based on Table 1, the comparison results show that smartphone camera digital numbers calculate the r-value greater than the r-table (0.4438). It can be said that the DN smartphone camera value is increasing. The value of nitrogen, phosphorus, and potassium will also increase. The regression tests can be carried out and used to determine the nutritional estimates of nitrogen, phosphorus, and potassium in rice crops. In contrast, chlorophyll has a low correlation value and negative results on the DN smartphone camera. Chlorophyll has a lower calculated r-value, so the correlation result value cannot be performed for regression tests.

The macronutrients (nitrogen, phosphorus, and potassium) in rice crops based on salinity indicators such as pH H₂O, EC (mS/cm), ESP, and SAR have processed the analysis of crops and soil samples in rice crops in the field. Based on Table 1, the comparison results show that the correlation between nitrogen, phosphorus, and potassium rice crops on the salinity indicators such as pH H₂O, EC, ESP, and SAR values is negative. It can be said that the higher the value of salinity indicators such as pH H₂O, EC, ESP, and SAR will be inversely proportional to nitrogen, phosphorus, and potassium rice crops, namely decreasing. In comparison, chlorophyll has a low correlation value for controlling pH H₂O, ESP, EC, and SAR indicators. Chlorophyll has a lower calculated r-value, so the correlation result value cannot be performed for regression tests.

Regression (R^2) parameters nitrogen, phosphorus, and potassium total biomass rice using smartphone camera digital number.

The R^2 value is obtained from the regression formula in nitrogen, phosphorus, and potassium crops, which means the data is accurate. The regression equation in Figure 6 shows that the y-axis nitrogen, phosphorus, and potassium total [%] in rice crops, and the x-axis shows the smartphone camera digital number (DN) value, so nitrogen, phosphorus, and potassium total in rice crops are affected by the smartphone camera DN value. In Figure 6, values 0.0035 (N), 0.0049 (P), and 0.0478 (K) are the slopes that determine linear regression direction and 0.8192 (N), -0.2042 (P), and -2.6717 (K) is intercept value. The slope value indicates a positive that the higher the x-values than the greater the y-value. The slope value also shows the rate of increase of nitrogen, phosphorus, and potassium total rice crops, an increase of nitrogen, phosphorus, and potassium the total rice crops increased by 0.0035 (N), 0.0049 (P), and 0.0478 (K). In contrast, the intercept value refers to the initial calculation value, when the values $x = 0$, then nitrogen, phosphorus, and potassium total rice crops are 0.8192 (N), -0.2042 (P), and -2.6717 (K), respectively.

The regression results have obtained the estimation of nitrogen, phosphorus, and potassium nutrients in rice crops. Nitrogen, phosphorus, and potassium data in rice crops in the field with estimated data nitrogen, phosphorus, and potassium using smartphone camera DN show values were not much different. The DN smartphone camera on each aerial photo after extraction produces pixels with red, blue, and green values converted in a DN value that can be used to guess the macronutrients of rice crops.

Accuracy assessment (t-pair test)

The estimation results using a smartphone were tested using a paired T-test to see the similarity of the laboratory data with the estimated N, P, and K nutrients for rice crops and salinity indicators. The estimated p-value is lower than $p > 0.05$, consisting of N, P, and K, which are 0.58, 0.81, and 0.97, respectively. The t-value also shows no difference between the results of laboratory analysis and estimates, which are -0.45 (N), 0.19 (P), and 0.11 (K), respectively. The paired t-test shows that the calculated t-value is smaller than the t-table value (0.68) for N, P, and K. This shows no difference between the results of macronutrient analysis from the labo-

ratory and the estimation results using a smartphone.

DISCUSSION

Smartphones can be used to identify macronutrient biomass in rice with an estimated value that does not differ from laboratory analysis results. DN smartphone can be used to predict N, P, and K crops. A study by (Amri & Sumiharto 2019) shows that a smartphone system can detect nitrogen, phosphorus, and potassium nutrients in rice fields in the Special Region of Yogyakarta. In addition, based on LPT Bogor research, the results of the detection of nutrient levels of nitrogen, phosphorus, and potassium showed an average detection accuracy of 70.65% (N 94.98%, P 50.84%, and K 66.14%). The best formula that results from the research results is $N_{Total} = 0.0035 * DN + 0.8192$ (R^2 0.84), $P_{Total} = 0.0049 * DN - 0.2042$ (R^2 0.70) and $K_{Total} = 0.0478 * DN - 2.6717$ (R^2 0.70), respectively (Figure 6).

Salinity affects the concentration of macronutrients in crops, reduces the accumulation of nitrogen in crops, phosphorus concentration, and decreases the accumulation of K^+ in crop tissues. This equation shows that every 1 unit increase in the

value of the DN Smartphone camera will have a positive effect on macronutrients (nitrogen, phosphorus, and potassium) of 83.7% (nitrogen), 70.34% (phosphorus), and 70.29% (potassium). In other words, the positive effect shows that the higher the DN value of the Smartphone camera, the more macronutrients (nitrogen, phosphorus, and potassium) increase. Jabon Regency has a fluvio-marine landform formed from a sedimentation process from a mixture of river sediment (alluvium) and marine sediment (marine). Jabon was previously an ocean that has become land so that the area still contains salt deposits in the underground part.

The metabolic imbalance caused by ion (Na^+) poisoning causes nutrient deficiency (nitrogen, phosphorus, and potassium). The destructive effect of salinity on plants is related to the high osmotic pressure of water, an imbalance between Na and K, Ca, Mg ions, and decreased uptake of nitrogen and phosphorus (Grattan & Grieve 1998). Salinity seems to affect two processes, namely water relations, and ionic relations. During initial exposure to salinity, the crops experience water pressure, which reduces the development of leaves. During long-term salinity exposure, crops experience ionic stress, causing three potential effects on crops: reducing water po-

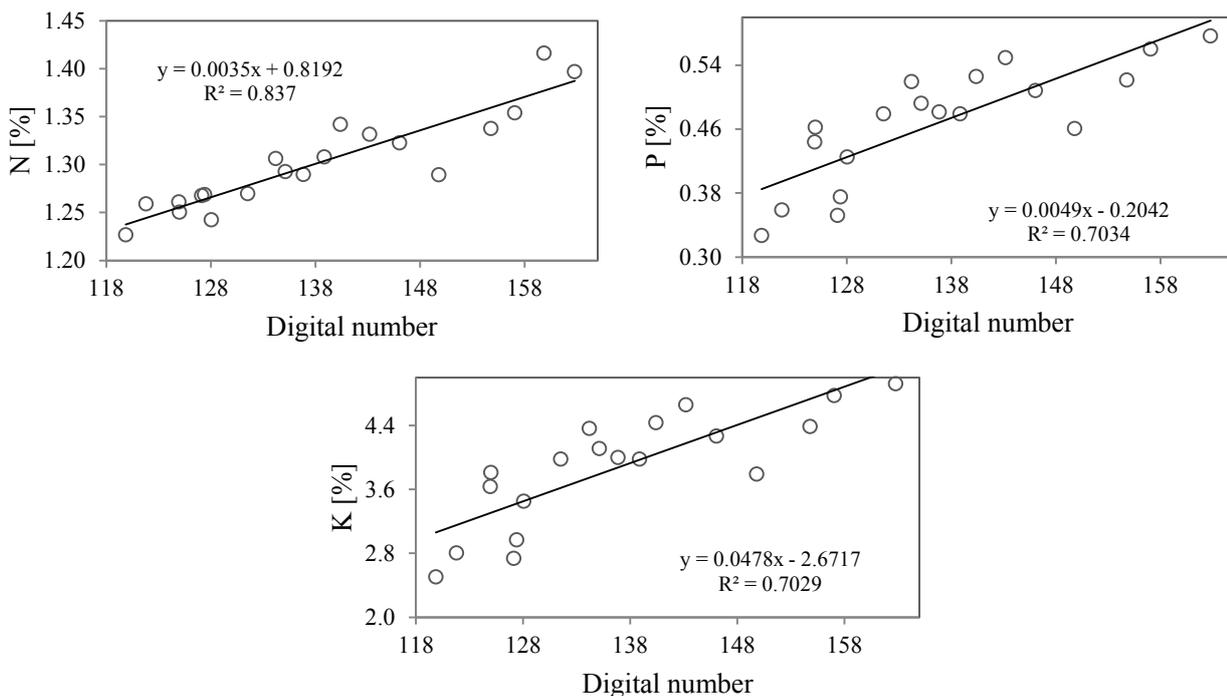


Figure 6. Regression graphic of nitrogen, phosphorus, and potassium total crops with DN smartphone camera
 Note: N – nitrogen; P – phosphorus; K – kalium

tency, direct toxicity of any absorbed Na and Cl, and disruption to the absorption of essential nutrients (Flowers & Flowers 2005). Salinity causes severe damage to many cellular and physiological processes, including photosynthesis, nutrient absorption, water absorption, root growth, and cell metabolism, which leads to decreased results (Darwish *et al.* 2009). Soil control affects the absorption of nitrogen, phosphorus, and potassium in crops. The specific effects of soil control on crop metabolism, EC in leaf aging, are associated with Na⁺ and Cl⁻ ion accumulation and decreased K⁺. Salinity associated with excess NaCl affects crop growth and yield by suppressing water and mineral absorption and normal metabolism (Al-Karaki 2000). According to Sipayung (2003), salinity inhibits the growth of roots, stems, and leaf area, as well as metabolic imbalances caused by ion poisoning (Na⁺) and nutrient deficiency (nitrogen, phosphorus, and potassium). The P concentration in agronomy crops in the field decreases with increased salinity. Salinity decreases P concentration in crop tissues; elsewhere, salinity

increases P or does not affect it. It is not surprising that differences between studies occur because P concentrations vary significantly in different experiments, and other nutritional interactions may coincide.

The increased salt concentration leads to the accumulation of toxic ions such as Cl⁻ and, in particular, Na⁺ in the cytosols. Several studies have shown that the concentration of K⁺ in crop tissue decreases along with increased salinity of NaCl. The decrease in K⁺ content in crops by Na⁺ is a competitive process. Salinity decreases the accumulation of K⁺ on leaves (Manchanda & Garg 2008). The adverse effect of salinity on crops is associated with high water osmotic pressure, the imbalance between Na ions with K, Ca, Mg. Moreover, it is also associated with decreased absorption of N and P (Grattan & Grieve 1998). In general, salinity reduces the accumulation of N in crops. This is because a decrease in nitrate concentration mainly accompanies the increase in absorption and accumulation of chloride (Garg *et al.* 1993).

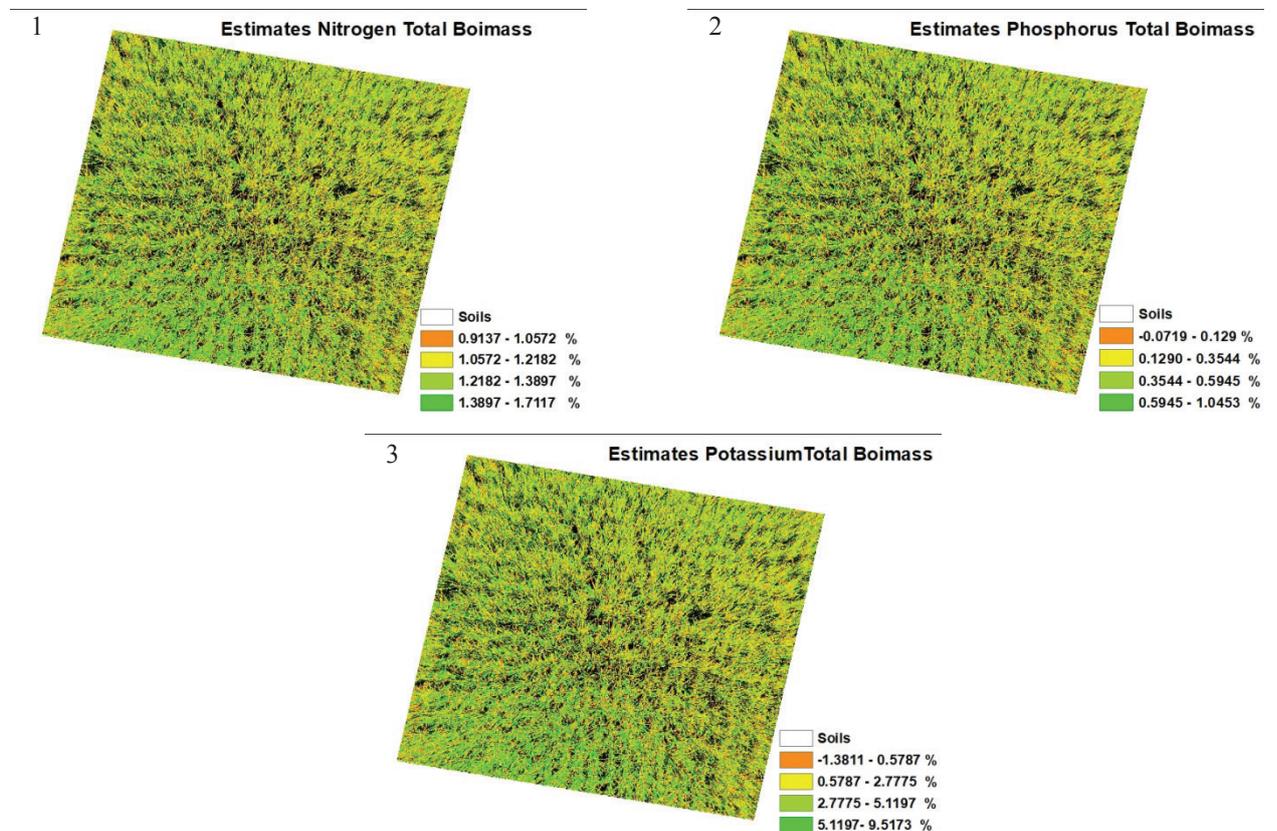


Figure 7. Results of aerial photo estimate nitrogen (1), phosphorus (2), and potassium (3) total biomass rice crops from the smartphone camera

The diversity of soil salinity is affected by the influence of the source of soil salinity, namely seawater in coastal areas. Thus, the observation point 1a to 10a and 1b to 10b describe salinity variability from low to high. Marwanto *et al.* (2009) explained that the closer to the saline source (coastal), the salinity increases. The digital signal processing is obtained by making use of the characteristic wavelength of the reflected leaves. The smartphone camera sensors can measure, analyse, monitor a condition, and then 'r' EC and changes in its surroundings. Several sensors on a smartphone camera include ambient light sensors, temperature, and humidity sensors (Maulana & Setiawan 2018). The smartphone camera is an active detection example that provides its energy source to illuminate targets and uses sensors to measure reflection energy by calculating the reflection angle or time required to recover energy. Some of the smartphone cameras utilize visible RGB (red, blue, and green) electromagnetic waves. Partially, RGB does not affect salinity; the digital number (DN) used is a combination of RGB. The results of the smartphone photo camera refer to the RGB image. The smartphone DN extraction value is obtained from the total RGB value in pixels (RGB combination). A combination of RGB values (red, green, and blue) of aerial photos combine the three primary colours, resulting in various colours in one (Santoso & Handoyo 2015).

The development of technology in various fields impacts digital image processing, one of them is on smartphones. Smartphones have many features, such as digital image capture (Budiman *et al.* 2019). A digital image is composed of a collection of dots called pixels to form a digital photo. Smartphones are operated by a Linux-based operating system that includes an operating system, middleware, and applications (Safaat 2011), more than 80% of smartphone users. Smartphones include operating systems with open source licenses that everyone can use freely to support daily activities and work, including in agriculture (Setiawan & Herdianto 2018). The use of smartphone cameras is one of the developments of satellite and drone imagery. The advantages are good image quality and can be arranged and easy to carry everywhere and the results are fast, there are menus such as brightness, sharpen, smooth, and edge detection (Adiyat 2013).

The most significant salinity factor in Jabon is the geographical position of Sidoarjo, which is on the seafront. Salinity as the most significant factor causes a decrease in nitrogen, phosphorus, and potassium in rice crops in Jabon. Meanwhile, other factors such as soil texture, plant varieties, and suboptimal management are less influential than the effect of salinity. Salinity affects soil texture, soil structure, and uptake of plant nutrients. With high salinity values, the availability of plant nutrients is deficient, so that the macronutrient of rice has decreased.

LIMITATIONS AND PROBLEMS IN THE FIELD

Aerial photos taken using a long stick with a smartphone camera are not used for large land due to the viewing angle. If the entire expanse of the rice field in the photo with a camera using a long stick, then some land will be photographed from the side. Aerial photography is affected by sunlight, brighter cross-sections, reflections of light from rice fields, and shadows in aerial photographs (such as a long stick). Taking photos needs to ensure good weather conditions. Avoid taking pictures when the sky is cloudy or the sun is scorching.

The pick-up is also affected by the area of the rice field map. As in transacts 2, observation points 1 and 2 have a small map width so that the outer part of the map is visible on the aerial photos. Choose a site with a large rice field map. Because of the remote location, it cannot be done for extrapolation to the other place. However, it can be overcome by the interpolation tool in ArcGIS software. One of them is Raster Calculator Tool using a math operation. This tool will calculate each pixel using an expression/algorithm (Rogers & Staub 2013). The smartphone camera device used in this study cannot connect with other smartphone cameras or remote control. It can be seen the accuracy of aerial photo-taking. So it uses a timer, then it must be repeated in lifting the long stick. Use a smartphone that supports remote control applications.

The variety and age of rice crops in the field vary considerably at each observation point, allowing variations in rice plants' appearance. The location is determined by trying to make the variety and age of the rice crop more uniform. The optimal age of rice crops used for shooting is in the vegetative phase 2 because it produces a better image (Mosleh

et al. 2015). In processing aerial photos using the smartphone camera, the extraction is still in composite data. Because it is done manually, the height is not standardized. Even though the gimbal has been installed, the shock when taking also affects the shooting results. A sampling at four corners and one center may be fewer DN and chlorophyll samples of rice crops. Aerial photo processing using a smartphone camera extraction can be done by programming to get a DN value that includes all pixels or can do orthophoto, then apply a zonal function. In taking aerial photos using a smartphone camera, the handling and height must be the same as the effective area and more stable in the shooting.

CONCLUSIONS

The content of soil macronutrients (nitrogen, phosphorus, and potassium) significantly affects the rice crop biomass. In saline soils, the availability of these macronutrients is reduced, problematic and hampered. Estimating macronutrient content using a terrestrial camera is an effort to identify nutrient deficiencies early. The macronutrient content has a strong effect on the digital number of smartphone camera values. It has a positive regression (nitrogen, phosphorus, and potassium) of 0.84, 0.70, and 0.70. This result means that the higher the saline indicator's value, the lower the nitrogen, phosphorus, and potassium values in rice crops. A smartphone camera (terrestrial) can monitor salinity stress's impact on reducing macronutrients (nitrogen, phosphorus, and potassium) in rice crops. The reliability test results of an aerial photo's digital number value through a smartphone camera have a smaller t-count value than the t-table. The estimated value is not significantly different from the actual data, so the aerial photo's digital number value through the smartphone camera can be applied to guess the macronutrients nitrogen, phosphorus, and potassium rice crops and salinity indicators.

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IMPROVEMENT OF WEEDS MANAGEMENT SYSTEM AND FERTILISERS APPLICATION IN WINTER WHEAT (*TRITICUM AESTIVUM* L.) CULTIVATION TECHNOLOGIES

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Wheat production plays a central role in the Russian agricultural system and significant land area is dedicated to this strategic crop. However, the wheat enterprise is highly constrained by weed interference which cause serious yield losses hence minimizing production income. The main objective of the study was to assess the efficacy of three various cultivation technologies as basic, intensive, and highly intensive systems on wheat biological efficiency. Three weed species *Echinochloa crus-galli*, *Stellaria media*, and *Viola arvensis*, and three winter wheat (*Triticum aestivum* L.) varieties Moscovskaya 40 (V1), Nemchinovskaya 17 (V2) and Nemchinovskaya 85 (V3) were studied. The data was analysed as a randomized complete block design with three replicates. Weed density, biological efficiency, yield performances, and selected qualitative parameters (measured through protein and gluten contents) were determined as affected by different cultivation technologies. The results showed that the high intensive cultivation technology (T3) was the most effective in reducing weed infestation levels as follows (0.3 plant/m² *Echinochloa crus-galli*, 0.5 plant/m² *Stellaria media* and 0.4 plant/m² *Viola arvensis*) with biological efficiency of 96%, while 81% and 90% were recorded with basic and intensive cultivation system respectively. Moreover, the highest wheat yield 10.6 t/ha was obtained by T3, with the greatest grain quality 5% higher than basic cultivation technology designated in T1. The results were variety-dependent revealing the intrinsic genetic performances and the different patterns of high competitive ability. The current results open real opportunities concerning the implementation of potent wheat production systems.

Key words: gluten content, herbicide, proteins content, weed, wheat varieties, yield

Chemical method plays a key role in controlling weeds that infest wheat fields. Herbicides are the most effective weed control tools developed recently in all over the world, suppressing 90–99% of weeds (Talgre *et al.* 2008). Nevertheless, herbicide resistance particularly in major field crops (wheat, rice, maize, soybean), has become a widespread problem posing a formidable challenge for global food production systems (Beckie *et al.* 2000; Ibrahim *et al.* 2016; Pansu *et al.* 2018). Currently many

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weed species are developing resistance to herbicides from different modes of action, and becoming more difficult and expensive to be controlled. Several studies in the recent years revealed that continued use of herbicides with the same mode of action (MOA) applies selection pressure on the weed population, this increases the genotype frequency of resistant individuals that eventually becomes the dominant component of the population (Mohammadi *et al.* 2018). As a solution, chemical management strategies such as rotating active ingredients with the different MOAs and combining (tank mix) herbicides from the various chemical families have been established to evade herbicide resistance. The selection of such herbicides should be based on biological knowledge of all dominant weed species in the field, while maintaining a balance between herbicide costs, weed thresholds, and environmental impacts (Nazarko *et al.* 2005; Chhokar *et al.* 2012).

In addition to the chemical methods, other control practices were developed to control weeds in farming systems. For example, increasing weed-crop competition, through the development of crop varieties with the high competitive ability can be illustrated. Previous studies reported that some crop genotypes had a high competitive ability against weeds (Mason *et al.* 2007; Fragasso *et al.* 2013). Hence, De Vita *et al.* (2017) investigated the effects of inter-row spacing as a cultural method on wheat competition against weeds. Mentioned study demonstrated that competitive ability directly depends on the wheat cultivars, thus, crop rotation was performed to control weed populations. It has been reported that crop rotation reduces growth, fecundity, and weed establishment by disrupting their life cycle (Nichols *et al.* 2015). Furthermore, it was observed that monoculture or continuous cropping within a field increases weeds density, whereas breaks in the cropping sequence with other crops often significantly diminish weeds interference (Hosseini *et al.* 2014).

Crop production for the purpose of human consumption such as wheat is of paramount importance worldwide (Polityko *et al.* 2020). Weeds are one of the major constraints causing serious losses in wheat yields and quality (Van der Meulen *et al.* 2017). Moreover, weed communities possess high phenological plasticity which enables them to adapt

to new management techniques (Vila-Aiub *et al.* 2019). Almost, direct yield losses due to weed infestations are ranged 20–40% in the global wheat production (Fahad *et al.* 2015; Gharde *et al.* 2018). For mentioned reasons, cropping systems need new management strategies, including cultural practices and genetic engineering technique (development of new varieties) to suppress weed growth and spread that cause wheat yield losses. The optimization of weed management systems, therefore, is becoming indispensable in wheat production systems to ensure high yields and better grain quality.

A three year study was carried out to investigate the effectiveness of three cultivation technologies included fertilisers, herbicides, and growth regulators in different combinations and doses to control weeds in order to estimate their influence on winter wheat yield and grain quality.

MATERIAL AND METHODS

Experimental site

Experiments were conducted over three wheat growing seasons (2016–2017, 2017–2018, and 2018–2019) at the Moscow Research Institute of Agriculture “Nemchinovka”, Odintsovo district, Russia (55° 45' N, 37°37' E and 200 m altitude).

Climatic conditions

The climate in the Moscow region was mid-continental characterized by mild winter, occasional flaw, and warm damp summer. The mean annual temperature was 6.3°C. The average temperature of the warm-season (May-October) was 13.5°C; the average monthly temperature in January and July was –8.40°C and 18.10°C, respectively. Positive ambient temperatures are experienced for an average of 215 days. Moreover, temperatures above 10°C (vegetation season) occur for an average of 130 days. The average cool season (November-March) temperature was –5.70°C. Mean annual precipitation was 628 mm: 56% in the spring-summer season and 26% in autumn. The average precipitation rate from May to September was 339 mm.

Weed density estimation

Weeds were investigated in three to five leaves stage of winter wheat. The dominant weed species

during three years of the experiment were: *Viola arvensis*, *Stellaria media* and *Echinochloa crus-galli*. These species typically germinate in autumn and spring in the condition of Moscow region. The weed density was calculated manually before treatments and after treatments (28 days after treatments). The weed density was expressed as number of plant/m² in each plot according to the cultivation technology. The total area of the experimental field was 700 m². The total area was sub-divided into three blocks; each block was further subdivided into nine plots in which the total area of each plot was 25 m². The experiment was performed in three replications for each treatment.

Experimental design and treatments

For three years of the study, the experiments were laid out as Randomized Complete Block Design with three replications. Three cultivation technologies [basic (T1), intensive (T2) and high intensive (T3)] were examined as the main plots whilst wheat varieties [Moscovskaya 40 (V1), Nemchinovskaya 17 (V2) and Nemchinovskaya 85 (V3)] were as sub-plots.

A description of experimental inputs for all three cultivation technologies is presented in Table 1. The herbicides were applied to wheat seedlings at the three-five leaves stage. The fertilisation was carried out in pre-sowing with top dressing, at the tillering and earing stages.

Sowing was done at the beginning of September with a planter (seeder SN 16 PM) at a rate of 5 million seeds/ha.

A modern combine harvester was used to harvest wheat (Polityko *et al.* 2020). The wheat was harvested around the mid-August at ripening stage.

The crop rotation implemented in the experimental field was as legumes, spring cereals and winter cereals. Particularly, the crop that preceded the winter wheat investigated in the study was peas.

Tillage operations were done before each growing season after harvesting the predecessor crop, with a ploughing depth of 20–22 cm.

Biological efficiency

The biological efficiency effectuated by the cultivation technologies was calculated manually 28 days after treatment and before harvest of winter wheat. Weed density was investigated and calculated as number of plant/m² in each experimental plot, according to the cultivation technologies applied, using the following formula (Polityko *et al.* 2020):

$$BE [\%] = 100 - (Nbr.a \times 100 / Nbr.b) \tag{1}$$

BE – biological efficiency [%]; Nbr.a – number of plant/m², after treatment; Nbr.b – number of plant/m², before treatment; 100 – conversion coefficient [%].

T a b l e 1

Applied herbicide and fertilisers in different cultivation technologies

Cultivation technologies	Fertilisers [kg/ha]	Crop protection details
1. Basic (T1)	Basal application N (30), P ₂ O ₅ (30), K ₂ O (90) [kg/ha] in pre-sowing and N (30) [kg/ha] at the tillering phase	• Herbicide: Lintur 180 [g/ha]
2. Intensive (T2)	Basal application N (60), P ₂ O ₅ (60), K ₂ O (120) [kg/ha] in pre-sowing, Top dressing, at the tillering phase, N (30) [kg/ha]	• Herbicide: Accurate Extra 25 [g/ha] • Growth regulator: Sapress 0.3 [L/ha]
3. High Intensive (T3)	Basal application N (90), P ₂ O ₅ (90), K ₂ O (150) [kg/ha] in pre-sowing, Top dressing, at the tillering and earing phases, N (30) and N (30) [kg/ha], respectively	• Herbicide: Accurate Extra 35 [g/ha] + Tandem 25 [g/ha] • Growth regulator: Sapress 0.3 [L/ha]

Determination of protein and gluten content

Selected qualitative parameters (determined via protein and gluten content) were measured for all winter wheat varieties.

The percentage of protein content was analysed by calculating the total nitrogen concentration in grain using the Kjeldahl method (Kjeldahl 1883), the following formula was used:

$$\text{Protein [\%]} = [(N \times 100) / (100 - W)] \times K \quad (2)$$

N – the nitrogen content in the grain [%]; W – the moisture content of the grain or its processed products [%]; K – conversion coefficient of nitrogen content to protein, equal to: 5.7 for wheat.

The gluten content in wheat grain was calculated by hand washing method (Polityko *et al.* 2020). The amount of raw or dry gluten in the grain %, calculated to the first decimal place, for dry-to the second decimal place by the following formula:

$$\text{Gluten [\%]} = \text{MG} / \text{Mg} \times 100 \quad (3)$$

MG – the number of raw or dry gluten [g]; Mg – the number of the sample of ground grain [g]; 100 – conversion coefficient [%].

Statistical analysis

Data analysis was performed using Statview 4.02 software (Abacus Concepts Inc., Berkeley, CA, USA). Values for each variable were expressed as the mean \pm SEM (Standard Error of Mean). Variables used for comparison purposes were the three weed species abundance as influenced by the three cultivation technologies: Basic (T1), Intensive (T2), and High Intensive (T3). Differences between treatments (wheat yield, gluten content, and proteins content) were assessed using Analysis of variance (ANOVA) at a significance of p -value < 0.05, and Tukey's test was used for mean comparisons in each treatment that was significant. Linear regression was used to evaluate the relationship between biological efficiency of cultivation technologies, wheat yield and grain quality.

RESULTS

Weed infestation

Figure 1 shows weed infestation of the three winter wheat species after treatments. Weed infestation levels were highest before treatment application as *Echinochloa crus-galli*, *Stellaria media* and *Viola arvensis* 30, 18 and 16 plant/m², respectively. However, after treatment, a significant weed reduction

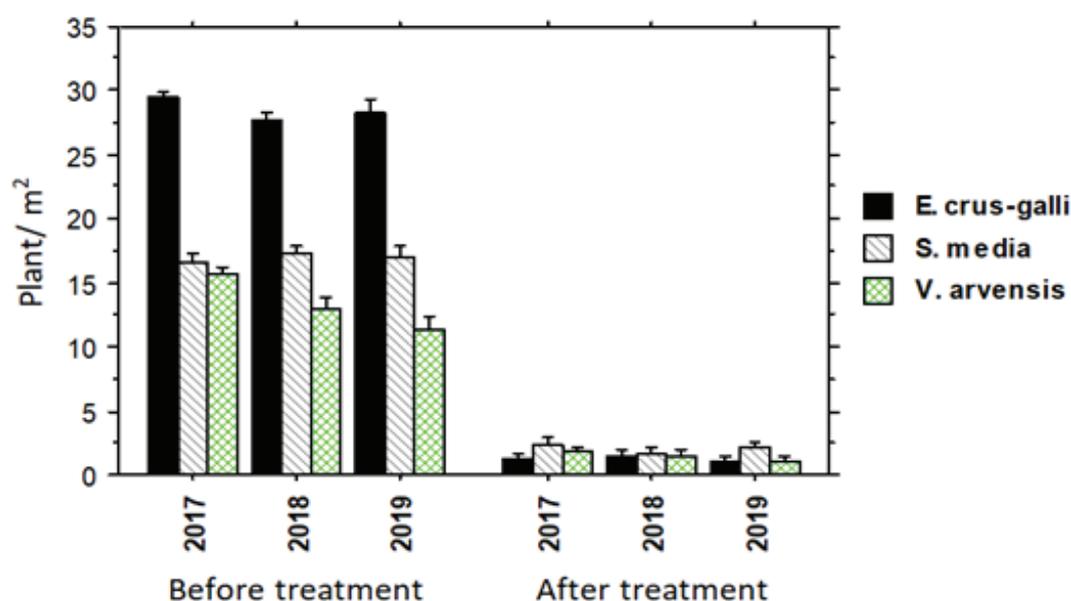


Figure 1. Influence of the cultivation technologies (T1 – basic, T2 – intensive and T3 – high intensive) on weed infestation levels of the three studied species

T a b l e 2

Grain yield and quality variation of each wheat varieties under different studied cultivation technology

Cultivation technology	Varieties	Year	Yield	Protein	Gluten
T1 – basic	Moscovskaya 40 variety (V1)	2017	9.5±0.23 ^b	14.1±0.05 ^c	30.4±0.11 ^c
		2018	5.2±0.05 ^d	16.4±0.05 ^b	32.8±0.08 ^b
		2019	7.8±0.20 ^{bc}	16.1±0.05 ^b	32.2±0.10 ^b
	Nemchinovskaya 17 variety (V2)	2017	9.7±0.11 ^{ab}	14.2±0.11 ^c	28.3±0.15 ^c
		2018	6.4±0.05 ^c	14.5±0.05 ^c	29.1±0.11 ^c
		2019	7.4±0.03 ^c	16.9±0.11 ^b	33.8±0.10 ^c
	Nemchinovskaya 85 variety (V3)	2017	9.9±0.28 ^b	14.8±0.05 ^c	31.2±0.05 ^c
		2018	7.1±0.06 ^c	15.1±0.05 ^c	30.2±0.10 ^c
		2019	7.4±0.03 ^c	17.6±0.11 ^b	35.2±0.15 ^{ab}
T2 – intensive	Moscovskaya 40 variety (V1)	2017	10.5±0.24 ^{ab}	15.4±0.05 ^b	31.5±0.05 ^c
		2018	8.4±0.11 ^b	17.7±0.05 ^b	36.7±0.15 ^{ab}
		2019	8.2±0.10 ^b	19.1±0.05 ^{ab}	38.2±0.15 ^a
	Nemchinovskaya 17 variety (V2)	2017	10.4±0.23 ^{ab}	15.6±0.10 ^b	33.2±0.08 ^b
		2018	7.1±0.14 ^c	16.1±0.05 ^b	32.2±0.15 ^b
		2019	7.9±0.11 ^{bc}	17.8±0.11 ^b	37.6±0.15 ^a
	Nemchinovskaya 85 variety (V3)	2017	13.1±0.17 ^a	15.6±0.05 ^b	32.5±0.05 ^b
		2018	7.3±0.05 ^c	16.4±0.05 ^b	32.8±0.10 ^b
		2019	8.3±0.05 ^b	18.9±0.11 ^{ab}	37.2±0.15 ^a
T3 – high intensive	Moscovskaya 40 variety (V1)	2017	14.1±0.17 ^{ab}	15.5±0.05	33.4±0.20 ^b
		2018	9.03±0.17 ^b	18.3±0.05 ^{ab}	36.6±0.10 ^{ab}
		2019	8.7±0.1 ^b	19.6±0.11 ^a	39.2±0.10 ^a
	Nemchinovskaya 17 variety (V2)	2017	13.3±0.17 ^a	16.8±0.05 ^b	35.1±0.05 ^{ab}
		2018	8.2±0.11 ^b	16.9±0.17 ^b	33.4±0.08 ^b
		2019	8.03±0.14 ^a	18.9±0.05 ^{ab}	39.8±0.05 ^a
	Nemchinovskaya 85 variety (V3)	2017	13.3±0.16 ^a	16.5±0.05 ^b	33.9±0.10 ^b
		2018	7.6±0.11 ^{bc}	16.8±0.05 ^b	33.6±0.10 ^b
		2019	8.7±0.05 ^b	19.4±0.11 ^{ab}	39.8±0.05 ^a
<i>p</i> -value	Cultivation technology		≤0.002	≤0.002	≤0.002
	Variety		≤0.003	≤0.002	≤0.003
	Year		≤0.002	≤0.004	≤0.002
	Cultivation technology × Variety		≤0.002	≤0.003	≤0.005
	Cultivation technology × Year		≤0.006	≤0.005	≤0.004
	Variety × Year		≤0.003	≤0.007	≤0.006
	Cultivation technology × Variety × Year		≤0.004	≤0.005	≤0.008

Values represent the average of 3 replicates ± SE (standard errors), *p*-values from ANOVA (cultivation technology, variety, year, cultivation technology × variety, cultivation technology × year, variety × year and cultivation technology × variety × year).

Different letters in column indicate significant difference between means and they were determined by Tukey test

value was observed for all three species. High intensive cultivation technology T3 determined as the most effective treatment in weed control, attaining 0.3 plant/m² *Echinochloa crus-galli*, 0.5 plant/m² *Stellaria media* and 0.4 plant/m² in *Viola arvensis*. Cultivation technologies 1 and 2 also showed high efficacy in reducing weed densities, since the weed control was ranged 75–80% and 85–93%, respectively.

Figure 2 exhibits the effects of the three applied cultivation technologies during the study (2017,

2018, and 2019). Cultivation technology (T3, high intensive) was the most effective in controlling weeds during the trial, since the weed density decreased over the years as shown by the following values 0.6 plant/m² ± 0.02, 0.3 plant/m² ± 0.02, and 0.2 plant/m² ± 0.01 in 2017, 2018 and 2019, respectively.

The biological efficiency of the investigated cultivation technologies is shown in Figure 3. The highest biological efficiency was obtained when cultivation technologies 2 and 3 were used with

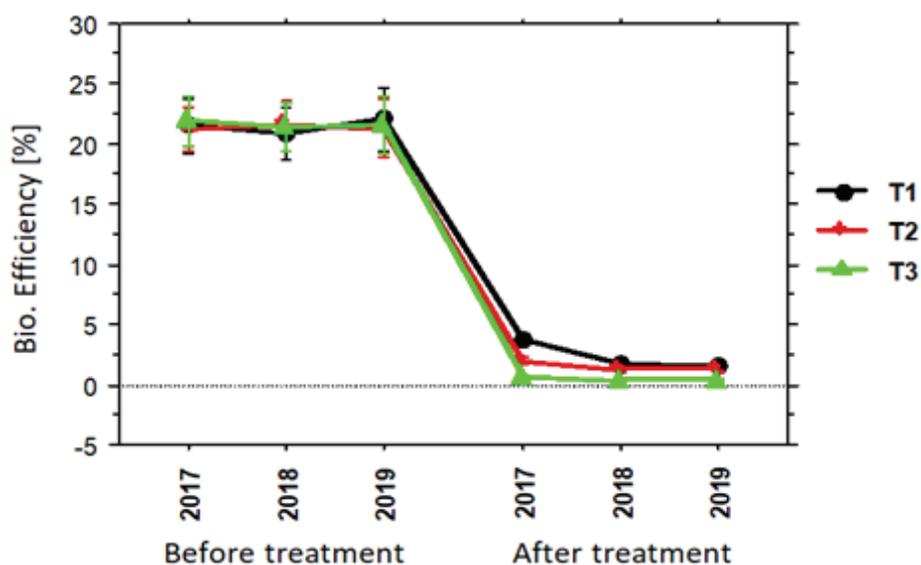


Figure 2. Effect of the three cultivation technologies (T1 – basic, T2 – intensive and T3 – high intensive) on biological efficiency during the study period (2017, 2018 and 2019)

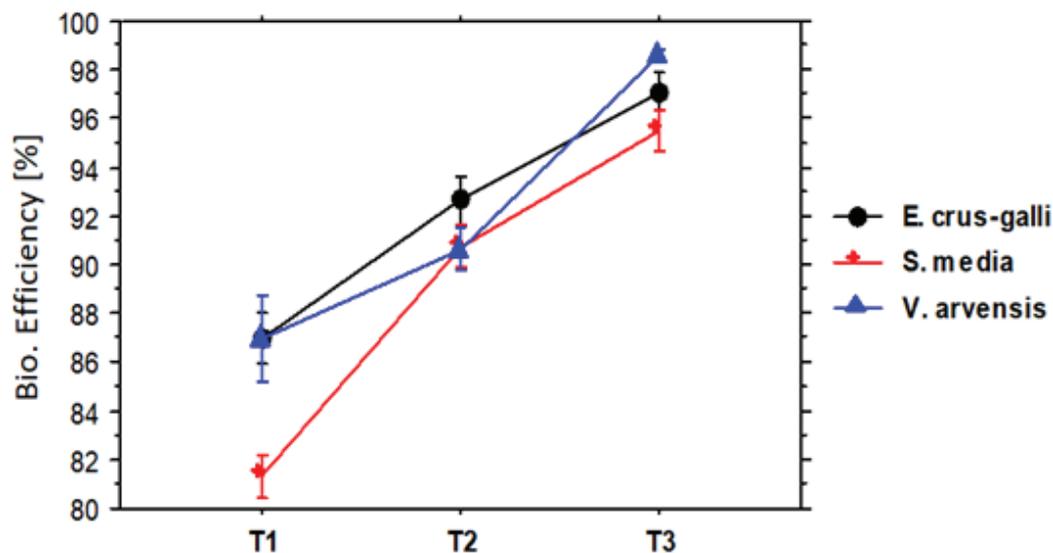


Figure 3. The biological efficiency of the investigated cultivation technologies to control weed (T1 – basic, T2 – intensive and T3 – high intensive)

90% and 96%, respectively. The cultivation technology 1 also seems to be effective in suppressing weeds, since 87, 81, and 88% weed biomass was reduced in *Echinochloa crus-galli*, *Stellaria media*, and *Viola arvensis*, respectively.

Yield performance and grain quality

Figure 4 displays yield performances of the three winter wheat varieties attributed to the cultivation technologies. High intensive cultivation technology

(T3) gave the best yields whatever the considered wheat variety. It is noted that there was no significant difference in the yield potential of all studied varieties. Moscovskaya 40 variety (V1), Nemchinovskaya 17 variety (V2) and Nemchinovskaya 85 variety (V3) yielded 10.6 ± 0.14 t/ha, 9.8 ± 0.39 t/ha and 9.9 ± 0.21 t/ha respectively throughout the study. On the other hand, the lowest yield was recorded when the basic cultivation technology was

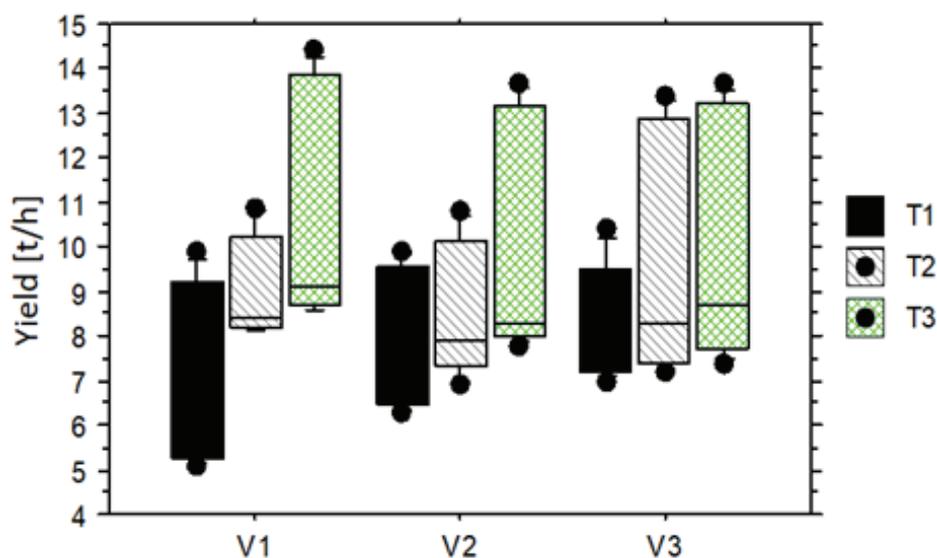


Figure 4. Yield performances of the three studied winter wheat varieties (Moscovskaya 40 variety (V1), Nemchinovskaya 17 variety (V2), and Nemchinovskaya 85 variety (V3)) as influenced by the cultivation technologies (T1 – basic, T2 – intensive and T3 – high intensive)

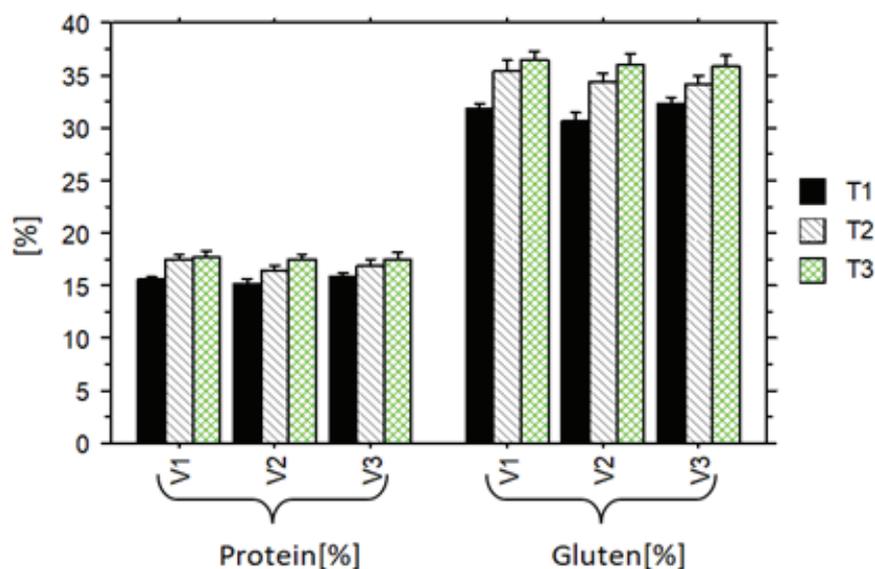


Figure 5. Protein and gluten contents of the three studied winter wheat varieties (Moscovskaya 40 variety (V1), Nemchinovskaya 17 variety (V2), and Nemchinovskaya 85 variety (V3)) attributed to the cultivation technologies (T1 – basic, T2 – intensive and T3 – high intensive)

implemented (T1) in all wheat varieties 7.5 ± 0.45 (V1), 7.8 ± 0.29 (V2) and 8.1 ± 0.27 t/ha (V3).

Grain quality was analysed based on protein and gluten contents in the different wheat varieties. The results showed that the protein and gluten content increased significantly by intensity of cultivation technologies (Figure 5). The highest protein and gluten content were observed when the high intensive cultivation technology was performed (T3) for all tested varieties in the values $17.8\% \pm 0.30$ (V1), $17.5\% \pm 0.32$ (V2) and $17.6\% \pm 0.42$ (V3) for protein content and $36.4\% \pm 0.90$ (V1), $36.1\% \pm 0.59$ (V2) and $36.7\% \pm 0.63$ (V3) for gluten content.

In Table 2, the analysis of variance for grain yield and quality (protein and gluten content) revealed that the cultivation technology, variety, and year had a highly significant effect on the yield and grain quality ($p \leq 0.05$). In addition, the yield and grain quality were significantly affected by the interaction between all studied treatments (cultivation technology \times variety, cultivation technology \times year, variety \times year, and cultivation technology \times variety \times year) ($p \leq 0.05$).

Figure 6 shows the relationship between biological efficiency of treatments, wheat yield, and grain quality by using linear regression. The biological efficiency of cultivation technologies is not correlated with grain yield $r^2 = 0.05$, however protein and gluten contents enhance with increasing biological efficiency of treatments $r^2 = 0.50$ and $r^2 = 0.52$, respectively.

DISCUSSION

Weeds are a serious challenge in wheat production. They generate colossal yield losses through the competition for nutrients with crops. An effective weed management system in modern agriculture is indispensable for the achievement of high grain yield. Here we demonstrate the practicability of varied levels of cultivation technologies in enhancing crop competitive ability against the weeds, hence, minimizing crop losses and simultaneously augmenting grain quality. Precursory studies evaluating the effect of herbicides and fertilisers in controlling

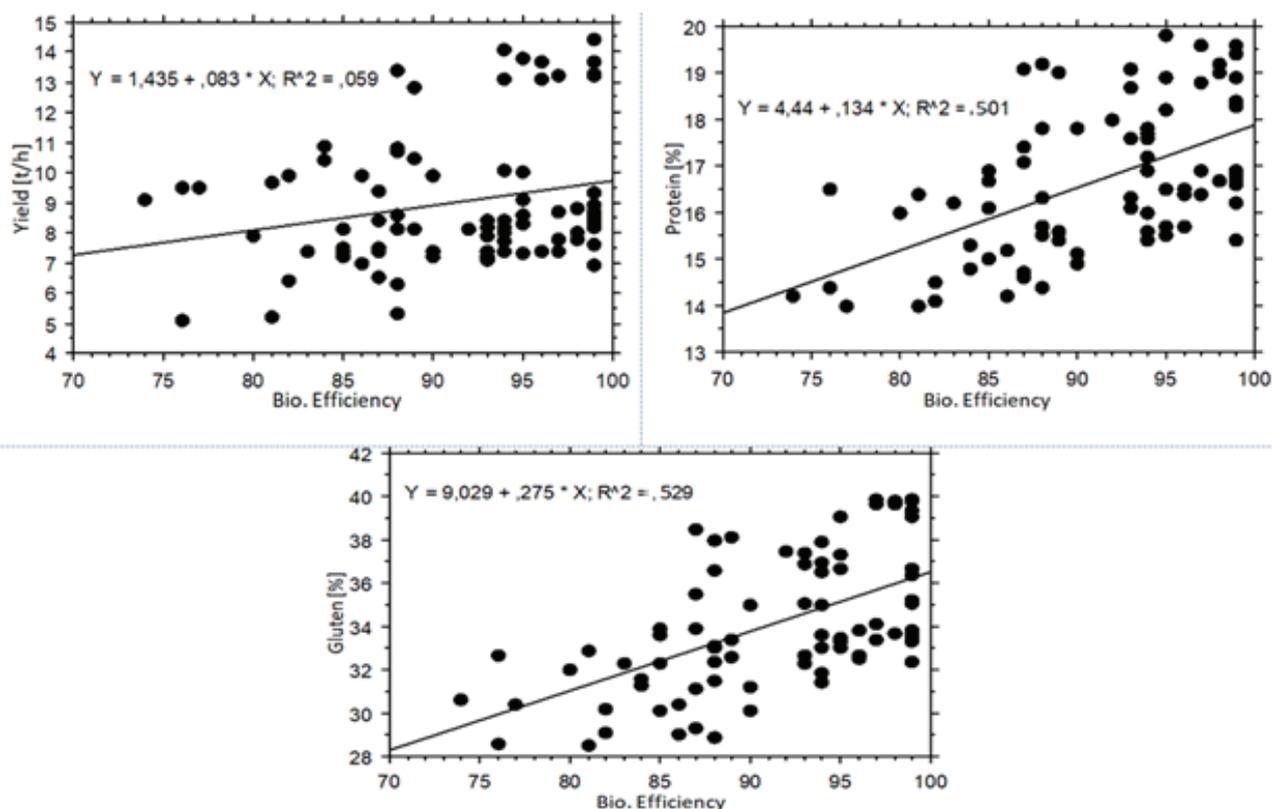


Figure 6. The relationship between biological efficiency of cultivation technologies, wheat grain yield, protein and gluten contents

weeds have illustrated that compliance with the doses of inputs and timely application improve wheat yield and grain quality (Latati *et al.* 2019; Rebouh *et al.* 2019).

Herbicides have become one of the most used methods for weed control. The present study was designed to determine the effect of three cultivation technologies, which also embraced the use of herbicides to control weeds in the winter wheat crop. The results showed that the studied cultivation technologies had favorable biological efficiency to control *Echinochloa crus-galli*, *Stellaria media* and *Viola arvensis*. The high intensive cultivation technology was the most effective among all experimental treatments, since 87, 81 and 88% efficiency was obtained for *Echinochloa crus-galli*, *Stellaria media* and *Viola arvensis* suppression, respectively. This was likely due to the presence of florasulam and tribenuron-methyl belonging to triazolopyrimidine class, which impedes the biosynthesis of branched-chain amino acids (leucine, isoleucine and valine) by inhibiting the enzyme ALS also known as AHAS (Weis *et al.* 2008).

Baghestani *et al.* (2007) and Zand *et al.* (2010) reported that florasulam and tribenuron-methyl were very effective for the management of *Echinochloa crus-galli*, *Stellaria media* and *Viola arvensis*, their findings are in agreement with those reported in the current study. High intensive cultivation technology including florasulam and tribenuron-methyl (Tandem), belonging to triazolopyrimidine, significantly reduced weed density 1.8 plant/m², 1.9 plant/m² and 1.2 plant/m² for *Echinochloa crus-galli*, *Stellaria media* and *Viola arvensis*, respectively.

Murphy and Lemerle (2006) have shown that the type and rate of fertilisers used play an important role in weed population shifts and an inappropriate dose of fertilisers significantly affects the abundance of weeds in crops. For example, the number of weed individuals decreases and their biomass increases with enhancing nitrogen fertiliser levels (Mahn *et al.* 1988; Tang *et al.* 2014).

In the current study, the implemented fertiliser rates [N (60–150), P₂O₅ (30–90), K₂O (90–150)] were balanced which promoted wheat crop growth, resulting in closed crop stands and limited light for weed communities. In addition, winter wheats (Moskovskaya 40, Nemchinovskaya 17 and Nem-

chinovskaya 85) seem to have the high competitive ability, which resulted in reduced weed pressure and diffusion in the cropping system. These results corroborate the findings of a great deal of the previous works, which demonstrated the importance of the competitive ability of the cultivated genotypes in weed management (Bastiaans *et al.* 2008; Andrew *et al.* 2015).

Weed resistance to herbicides is increasingly becoming problematic in crop production systems across the globe. The repeated use of herbicides with the same mode of action ultimately leads to the rapid spread of the resistant genotypes in the weed population (Heap *et al.* 2018; Nakka *et al.* 2019). The established spatio-temporal analysis to study the effect of cultivation technologies on weed species abundance carried out over three years of trials (Figure 2) showed that the investigated cultivation technologies reduced weeds infestation levels over the years. This is probably due to the combined effect of crop rotation and tillage before the sowing season, which optimized the efficacy of herbicides in weed control. These results were in consistent with the findings reported by Mishra and Singh (2012), whereby tillage followed by herbicide application increased biological efficiency and reduced the chances of the weeds developing resistance to applied herbicides. Messaoudi *et al.* (2020) and MacLaren *et al.* (2021) also noticed that the combined tillage-crop rotation provides better weed control results than tillage-monoculture combination, which leads us to conclude that weed management depends on several factors that constitute cultivation technology such as herbicide, crop rotation, tillage, fertilisation, genetic characteristics of cultivars and others.

Previous studies demonstrated that herbicide use generally does not affect wheat yields, but increases grain quality of crops (Storkey *et al.* 2003; Benjamin *et al.* 2010; Gaba *et al.* 2016). This study was premised on assessing the efficacy of the herbicides both in weed control and enhancement of wheat yield and the grain quality. The analyses of the relationship between biological efficiency of treatments, wheat yield and grain quality by using the linear regression did not show any relationship between wheat yields and herbicide use ($r^2 = 0.05$), however, there is a moderately positive relationship

between protein, gluten content, and biological efficiency of herbicide $r^2 = 0.50$, $r^2 = 0.52$, respectively (Figure 6). This is probably due to the competitive ability of the cultivated genotypes, which increases their nutrient uptake rate, thus improving grain quality (Van der Meulen *et al.* 2017; Polityko *et al.* 2020). Moreover, our findings from the previous study where we explored the nutrient use efficiency of the winter wheat cultivars investigated in the current work exhibited that Moskovskaya 40, Nemchinovskaya 17 and Nemchinovskaya 85 had high nutrient uptake capacity (Rebukh *et al.* 2019).

CONCLUSIONS

Weeds are one of the major constraints in crop yields and quality, causing huge economic losses. The present study showed that the weed management systems by the using several factors including fertilisers and growth regulators, at indicated rates, offered optimal weed reduction (96%) and increased grain yield by 3 t/ha. Moskovskaya 40, Nemchinovka 17 and Nemchinovka 85 expressed high competitive ability. Thus, the obtained results in our study indicated that balanced fertilisation and rational use of herbicides could be used as an effective method to suppress weeds, which allows not only maintenance of a stable crop yield but also greatly reduce weed reproduction. The current results open real opportunities for large-scale performing the cultivation technologies in different regions of Russia and exhibited new varieties that could offer high performance and weed-crop competitive ability.

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GRAIN AND OIL YIELDS OF SAFFLOWER (*CARTHAMUS TINCTORIUS* L.) AFFECTED BY WATER DEFICIT AND GROWTH REGULATORS

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Farzi-Aminabad, R., Ghassemi-Golezani, K. and Nasrullahzadeh, S. (2021). Grain and oil yields of safflower (*Carthamus tinctorius* L.) affected by water deficit and growth regulators. *Agriculture (Pol'nohospodárstvo)*, 67(2), 87–94.

In order to evaluate the effects of growth regulators on yield parameters and oil content of safflower (*Carthamus tinctorius* L.), a field experiment was conducted under different irrigation intervals in 2019. All plots were irrigated regularly until the seedling establishment and thereafter irrigation intervals were applied after 70, 100, 130, and 160 mm evaporation from class A pan, as normal irrigation and mild, moderate, and severe water deficits, respectively. Foliar sprays of water (control), putrescine (60 µg/L), and 24-epibrassinolide (25 µg/L) at a rate of 1,000 L/ha were applied slightly before flowering. The results revealed that means of plant biomass, grains per capitulum, grains per plant, grain yield, harvest index, oil percentage, and yield were decreased under limited irrigations, but 1,000-seeds weight was only reduced under severe water deficit. However, foliar sprays of growth regulators, particularly putrescine, increased grains per plant, grain yield, and harvest index, leading to an improvement in oil yield per unit area under different levels of water supply. These results suggest that foliar application of putrescine is a superior treatment for improving the productivity of safflower plants under normal and stressful conditions.

Key words: drought stress, harvest index, epi-brassinolide, plant biomass, putrescine

Safflower (*Carthamus tinctorius* L.) is originated from south Asia and is cultivated in the same regions that favor the growth and development of wheat and barley. It is undoubtedly a crop with great potential and compatibility to be grown in a wide range of environments (Gilbert *et al.* 2008). Nevertheless, the productivity of this crop could be limited by abiotic stresses such as water deficit. Drought is one of the most detrimental environmental stress for plant growth and development (Krouma *et al.* 2015), that can affect different physiological and biochemical processes in plants (Mohammadi *et al.* 2018a). Water deficit can naturally occur in the field when water

availability is less than evapotranspiration demand. This can cause a decline in carbon assimilation, cell growth, and tissue expansion. Many genes, enzymes, hormones, and metabolites are involved in each of these processes (Skirycz & Inze 2010).

Putrescine as a polyamine is involved in scavenging of the free radicals and regulating osmotic potential under drought stress (Gupta *et al.* 2013; Li *et al.* 2014). It can stimulate plant growth by acting as a nitrogen source and influencing cell division and development (Kandil *et al.* 2011). There is also a relationship between brassinosteroids (BRs) concentration and oxidative stress in plants (Bajguz

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& Hayat 2009). Some reports revealed that exogenous application of BRs such as 24-epibrassinolide alter the activities of antioxidant enzymes such as catalase, superoxide dismutase, ascorbate peroxidase, and glutathione peroxidase under stressful conditions (Ozdemir *et al.* 2004). These are a group of steroidal growth regulators (Bajguz & Piotrowska-Niczyporuk 2014) influencing stress tolerance, seed germination, growth, and senescence of plants (Rao *et al.* 2002). This research was aimed to assess the impact of exogenously applied putrescine and 24-epibrassinolide on reducing the harmful effects of drought stress on field performance of safflower plants.

MATERIAL AND METHODS

A field experiment was conducted in 2019 at the Research Farm of the University of Tabriz in Iran, located at 38.05° N, 46.17° E with an altitude of 1,360 m above sea level, annual rainfall about 263 mm, and mean annual temperature of 13.5°C. The annual rainfall during 2017–2020 ranged from 200.87 mm up to 380.75 mm. The total rainfall during experimentation was about 7 mm and the mean temperature ranged 15.7–29.8°C. The physical and chemical properties of the farm soil are presented in Table 1.

The experiment was laid out as a split plot based on randomized complete block design in three replicates with irrigation intervals (I₁, I₂, I₃, and I₄: irrigation after 70, 100, 130, and 160 mm evaporation from class A pan, for normal irrigation and mild, moderate and severe water deficits, respectively) in main plots and foliar spray of growth regulators in sub-plots. Each plot consisted of 6 rows with 5 m

length, spaced 25 cm apart. The seeds were sown manually on 14th May 2019 at a depth of 3–4 cm, with a distance of 10 cm on the rows to achieve a density of 40 plants/m². All plots were irrigated after sowing. Subsequent irrigations were carried out according to the treatments up to field capacity (FC). Weeds were controlled by hand during plant growth and development. Variants of foliar sprays of growth regulators: water (control), putrescine (60 µg/L), and 24-epibrassinolide (25 µg/L) at a solution rate of 1,000 L/ha were applied on plants slightly before flowering. At maturity, plants in 1 m² of the middle part of each plot were harvested and the number of grains per capitulum, number of grains per plant, 1,000-seeds weight, and grain yield were determined. Aboveground biomass was oven-dried at 75°C for 48 hours and then, plant biomass per unit area was recorded. Harvest index was calculated as:

$$\text{Harvest index} = (\text{grain yield}/\text{plant biomass}) \times 100$$

A Soxhlet extractor was used to extract oil according to the AOCS method (1993). Initially, 5 grams of grains from each plot were crushed by a milling machine. The filter papers were cut into bottom tubes and the shredded samples were poured into paper tubes and placed inside the Soxhlet extractor. 200 ml of petroleum ether was poured into a round bottom flask and a few pieces of boiling stone were thrown into it. The Soxhlet extractor and refrigerant were then installed on the flask. Then the faucet was turned on to allow water to flow into the refrigerant. As soon as the first drop of solvent was distilled and dripped from the refrigerant, the time was recorded. Extraction was continued for 5 hours. The heat source was then turned off to cool the system, and the vapor was cooled to a liquid. After that, the flask was removed from the clamp.

T a b l e 1

Physical and chemical properties of farm soil

Depth [cm]	pH	Electrical conductivity	Total neutralizing material [%]	Organic carbon [%]	Total nitrogen [%]	Absorbable elements [mg/L]						Mineral components of soil [%]			Soil texture
						P	K	Ca	Fe	Zn	Mn	Clay	Silt	Sand	
0–30	8.0	2.92	10.5	0.37	0.04	4.9	255	0.76	2.6	0.92	3.34	12	14	74	Sandy loam

The samples were then weighed and oil percentage and yield were determined.

The data were analyzed using the model of two-factorial analysis of variance (irrigation interval, growth regulators), by MSTATC software; and the means were compared by Duncan multiple range test at $P \leq 0.05$. The Excel software was used to draw figures.

RESULTS AND DISCUSSION

Analysis of variance (Table 2) showed that water deficit and growth regulators had significant effects on plant biomass, number of grains per capitulum, number of grains per plant, 1,000-seeds weight, grain yield, harvest index, and oil yield. The oil percentage was also significantly affected by water limitation. The interaction of irrigation \times growth regulator was significant for grains per plant, grain yield, harvest index, and oil yield.

The plant biomass was decreased with increasing irrigation intervals (Table 3). Foliar spray of growth regulators increased plant biomass of safflower, with no significant difference between putrescine and 24-epibrassinolide treated plants (Table 3). As determined by Ghassemi-Golezani and Afkhami (2018), plant biomass reduction in water stressed plants is the consequence of decreasing cell growth,

leaf area, and intensity of photosynthesis. Water deficit decreases the water potential of plants, leading to stomata closure and reduction in photosynthesis rate, leaf growth (Ozturk 1999), and plant biomass. Reduction in plant biomass resulted in decreasing the number of grains per plant, 1,000-seeds weight, and consequently, grain yield and harvest index of milk thistle (Ghassemi-Golezani *et al.* 2017). Decreasing plant biomass by water limitation has also been observed in sesame (Eskandari *et al.* 2009) plants. The increment of plant biomass in putrescine-treated safflower plants was related to further expansion and persistence of leaf area, providing sufficient photosynthates for plant growth (Emadi *et al.* 2013). Application of brassinolide can also enhance plant dry matter through increasing relative water content, and chlorophyll content in soybean (Zhang *et al.* 2008).

The number of grains per capitulum was decreased under water deficit, with no significant differences among limited irrigations (Table 3). The highest number of grains per capitulum was recorded for putrescine treated plants, with no significant difference between growth regulators (Table 3). Foliar application of brassinolide also increased the number of grains per spike of wheat under normal and limited irrigations (Dehghan *et al.* 2017). This growth regulator increased the number of flowers and grain yield in mung bean, due to the prevention of flower

T a b l e 2

Analysis of variance (mean squares) of grain yield and yield components in safflower affected by water deficit and growth regulators

Source of variation	df	Plant biomass	Grains per capitulum	Grains per plant	1,000 seeds weight	Grain yield	Harvest index	Oil percentage	Oil yield
Replication	2	4954.40	91.29	799.36	5.04	30.99	4.090	0.029	2.73
Irrigation (I)	3	117,307.33 ⁺⁺	77.69 ⁺⁺	19,586.49 ⁺⁺	5.70 ⁺	29,794.93 ⁺⁺	453.57 ⁺⁺	30.20 ⁺⁺	3,191.49 ⁺⁺
E _a	6	1,002.59	15.78	460.47	0.78	322.08	15.76	0.19	19.74
Regulators (R)	2	26,393.44 ⁺⁺	303.32 ⁺⁺	11,587.68 ⁺⁺	4.36 ⁺	15,479.56 ⁺⁺	70.14 ⁺⁺	0.017 ^{ns}	1,147.94 ⁺⁺
I \times R	6	142.10 ^{ns}	3.28 ^{ns}	364.39 ⁺	0.90 ^{ns}	504.02 ⁺⁺	9.35 ⁺	0.033 ^{ns}	56.80 ⁺⁺
E _b	16	362.65	4.17	112.75	0.80	116.38	2.26	0.033	10.63
CV [%]	–	3.02	7.12	6.29	3.23	5.79	2.67	0.68	6.47

E_a, E_b – errors for main and sub-plots, respectively; CV – coefficient of variation; df – degrees of freedom
⁺, ⁺⁺ and ^{ns} – significant at $p \leq 0.05$, $p \leq 0.01$ and no significant (F-test), respectively

abortion and higher content of phenolic compounds (Ananthi *et al.* 2013). Foliar application of brassinolide increases the transport of nutrients within the plant (Fuji *et al.* 1991). Brassinolide is involved in physiological and metabolic processes such as photosynthesis, nucleic acid synthesis, proline accumulation, and protein production, as well as in the gene transcription and translation, leading to an increase in protein content, including enzymes (Anjum *et al.* 2011).

The number of grains per plant significantly decreased with increasing irrigation interval, without significant differences between mild and moderate stress. Foliar sprays of growth regulators led to a significant increase in the number of grains per plant at all levels of water stress. A significantly higher value was recorded in the variant with putrescine compared to brassinolide only under normal irrigation and severe stress (irrigation interval × growth regulator interaction). This superiority was more pronounced under normal irrigation (Figure 1). The decrement in the number of grains per plant under water limitation was associated with the reduced number of grains per capitulum (Table 3). Ghassemi-Golezani and Afkhami (2018) found that the main reason for a decline in the number of grains per plant under water deficit was the reduction of green cover percentage and plant biomass. Reduction in

the number of grains per plant due to water deficit is also reported in chickpea (Ghassemi-Golezani *et al.* 2008), sesame (Eskandari *et al.* 2009), and milk thistle (Ghassemi-Golezani *et al.* 2017). This reduction may be caused by excessive loss of leaves at reproductive stages as reported in sunflower (Rauf 2008).

Increasing irrigation intervals decreased 1,000-seeds weight, but there was no significant difference among normal irrigation, mild stress, and moderate stress (Table 3). Foliar application of both growth regulators increased 1,000-seeds weight (Table 3). Increasing grain weight by foliar spray of growth regulators could be the result of from prolonged leaf area duration and, hence, a longer grain filling period. Environmental stresses such as water shortage, especially during grain filling can reduce photosynthesis and remobilization of stored materials and hence, grain filling duration (Sadeghipour 2008). Water stress generally accelerates leaf senescence and shortens grain filling duration (Davis *et al.* 1999). Also, water deficit reduced 1,000-seeds weight and grain yield by shortening the grain filling period of wheat (Gooding *et al.* 2003). Reduced grain filling occurs due to decreased segregation of assimilates and activities of enzymes involved in sucrose and starch biosynthesis (Srivastava & Suprasanna 2015). Application of brassinosteroids also

T a b l e 3

Means of plant biomass, number of grains per capitulum and 1,000-seeds weight for different irrigation intervals and foliar spray of growth regulators

Treatment	Plant biomass [g/m ²]	Grains per capitulum	1,000-seeds weight [g]
Irrigation			
I ₁	765.1 ^a	32.9 ^a	27.9 ^{ab}
I ₂	655.4 ^b	27.8 ^b	28.4 ^a
I ₃	615.5 ^c	27.4 ^b	28.1 ^{ab}
I ₄	488.6 ^d	26.4 ^b	26.6 ^b
Foliar spray			
Water (control)	580.0 ^b	23.1 ^b	27.08 ^b
Putrescine	672.0 ^a	32.8 ^a	28.25 ^a
24-epibrassinolide	641.5 ^a	30.1 ^a	27.95 ^a

Different letters in each column indicate significant difference at $p \leq 0.05$ (Duncan test)

I₁, I₂, I₃, I₄ – irrigation after 70, 100, 130 and 160-mm evaporation from class A pan, respectively

increased grain weight and; consequently, eventually grain yield in chickpea (Shahid *et al.* 2011). Emam and Doghezloo (2015), reported that water stress has reduced grain weight and foliar application of brassinosteroids has increased grain weight in wheat cultivars under different irrigation variants.

Reduction in grain yield under water stress (Figure 2) can be attributed to reduced grains per capitulum (Table 3), grains per plant (Figure 1) and 1,000-seeds weight (Table 3). There is a significant positive correlation of 1,000-seeds weight and grain yield of safflower (Yari & Keshtakar 2016). Water limitation during flowering leads to flower abortion and poor seed set, which can potentially reduce grain yield of chickpea per unit area (Fang *et al.* 2009). The growth regulators, especially putrescine, enhanced the grain yield of safflower under normal and limited irrigations, with a greater impact in normal watering (Figure 2). Zafari *et al.* (2017), reported that safflower yield was reduced by water deficit, but it was improved by 24-epibrassinolide treatment through enhancing plant metabolism and stress tolerance (Talaat & Shawki 2013). Application of putrescine on drought-stressed wheat plants increases the number of spikes, spike weight; and grain yield (Gupta *et al.* 2012). Polyamines such as putrescine have antioxidant properties, which in-

hibit membrane lipid peroxidation and reduce the production of free radicals. It also prevents the synthesis of ethylene and delays the aging of the plant, thereby increasing the effective period of grain filling and grain yield (Bregoli *et al.* 2002).

Foliar application of the growth regulators had a significantly positive impact on the harvest index of safflower under all irrigation intervals. However, putrescine was more effective than 24-epibrassinolide, particularly under normal irrigation (Figure 3). This reflects the higher impact of growth regulators on grain yield (Figure 2) than on plant biomass (Table 3). The high harvest index is related to the formation of more grains per plant and allocation of more assimilates to the grains, which is strongly supported by a previous report on beans (Munoz-Pera *et al.* 2006). Increasing irrigation intervals caused a decline in bean harvest index, while foliar application of 24-epibrassinolide enhanced it (Mohammadi *et al.* 2018b). This improvement in harvest index could be resulted from the higher effect of 24-epibrassinolide on grain yield, compared to plant biomass.

The oil percentage of safflower grains was decreased with decreasing water availability (Figure 4). The oil content was mainly controlled by the genetic characteristics of a cultivar, but photosynthetic

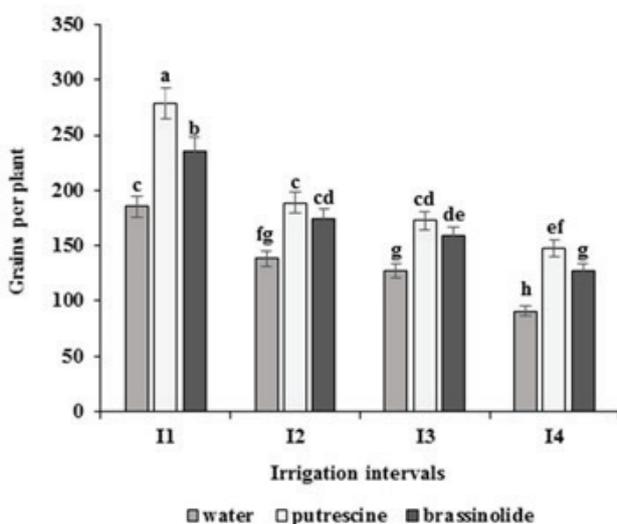


Figure 1. Means of safflower grains per plant for interaction of irrigation intervals × growth regulators
 – I1, I2, I3 and I4 – irrigation after 70, 100, 130 and 160-mm evaporation, respectively,
 – Different letters indicate a significant difference at $p \leq 0.05$ (Duncan test).

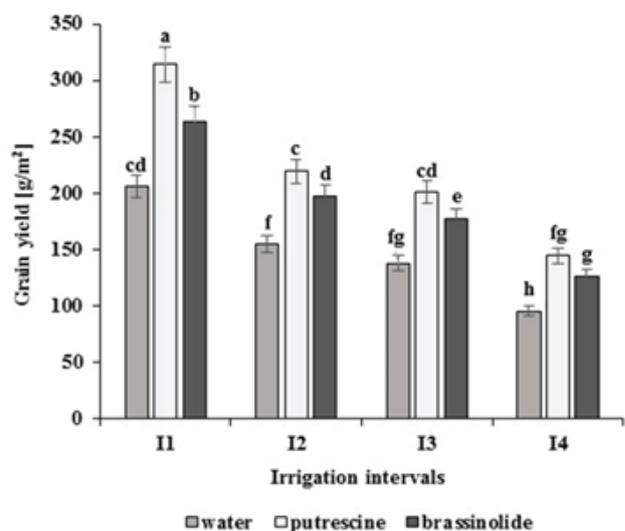


Figure 2. Means of grain yield for interaction of irrigation intervals × growth regulators in safflower
 – I1, I2, I3 and I4 – irrigation after 70, 100, 130 and 160-mm evaporation, respectively,
 – Different letters indicate a significant difference at $p \leq 0.05$ (Duncan test).

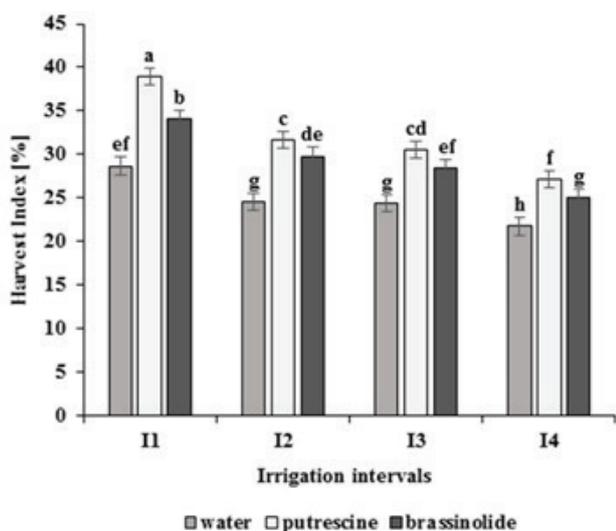


Figure 3. Means of harvest index for interaction of irrigation intervals × growth regulators in safflower – I1, I2, I3 and I4 – irrigation after 70, 100, 130 and 160-mm evaporation, respectively, – Different letters indicate a significant difference at $p \leq 0.05$ (Duncan test).

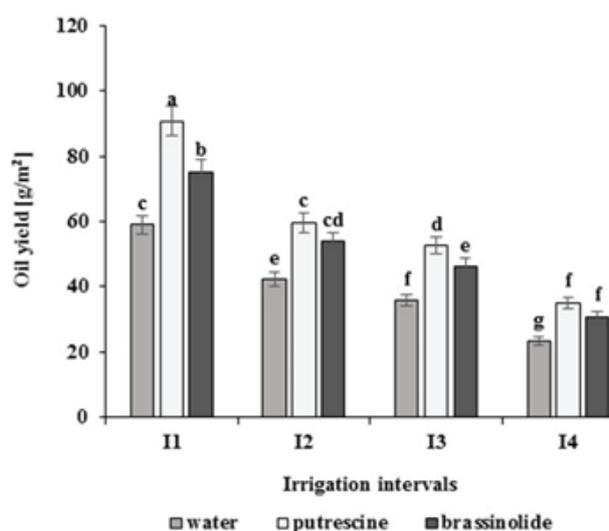


Figure 5. Means of safflower oil yield for interaction of irrigation intervals × growth regulators – I1, I2, I3 and I4 – irrigation after 70, 100, 130 and 160-mm evaporation, respectively, – Different letters indicate a significant difference at $p \leq 0.05$ (Duncan test).

products as the source of oil synthesis can be greatly reduced by water deficit. This might be also explained by decreased availability of carbohydrates for oil synthesis under drought stress (Ashrafi & Razmjou 2014). Mohammadi *et al.* (2018a) reported that a decline in oil percentage of safflower grains in water deficit conditions is related to the reduction of the seeds' capacity for oil accumulation and

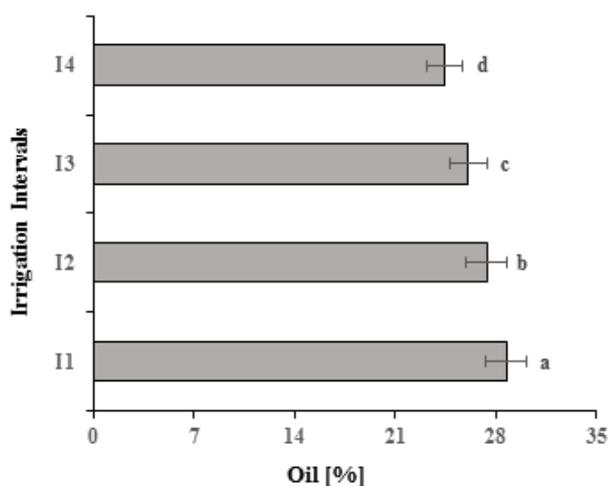


Figure 4. Changes in mean oil percentage of safflower seeds under different irrigation intervals – I1, I2, I3 and I4 – irrigation after 70, 100, 130 and 160-mm evaporation, respectively, – Different letters indicate a significant difference at $p \leq 0.05$ (Duncan test).

oxidation of the oil. Increasing irrigation intervals in maize also decreased the percentage of seed oil (Ghassemi-Golezani *et al.* 2016).

A significant reduction in oil yield of safflower with increasing irrigation interval was the result of decrements in grain yield (Figure 2) and oil percentage (Figure 4), which was also supported by previous reports on this crop (Ghassemi-Golezani & Afkhami 2018). Drought stress severely limits the growth and development of plants by affecting different metabolic processes such as CO_2 assimilation and oil and protein synthesis (Nasirkhan *et al.* 2007). Esmailian *et al.* (2012), reported that water stress at the grain filling stage of sunflower caused a decrease in oil content. There is a strong positive correlation between oil yield and grain yield of safflower (Omidy Tabrizi *et al.* 2000).

Foliar spray of putrescine was the superior treatment on increasing oil yield of safflower under normal and limited irrigations, followed by 24-epibrassinolide. This advantage was greater under normal irrigation, compared to other irrigation intervals (Figure 5). This superiority in oil yield was directly related to the positive impacts of 24-epibrassinolide and particularly putrescine on grain yield per unit area (Figure 2), since oil percentage was not affected by these growth regulators (Table 2).

CONCLUSIONS

Water limitation significantly reduced plant biomass, grains per capitulum, grains per plant, 1,000-grain weight, grain yield, and harvest index of safflower, which were improved by foliar spray of growth regulators. Oil percentage and yield of safflower grains were also decreased with decreasing water supply. Application of putrescine and 24-epibrassinolide noticeably enhanced grains per plant, grain yield and harvest index, leading to an improvement in oil yield per unit area under all irrigation intervals, especially under normal irrigation. Foliar spray of putrescine was the superior treatment for improving these traits. Therefore, application of 24-epibrassinolide and especially putrescine can considerably enhance safflower plant productivity under normal and limited irrigations.

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