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Review

Potential use of rhizobium for vegetable crops growth promotion

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The system of vegetable crops production required large amounts of mineral fertilizers. One of the possible alternatives to assure the economic and environmental sustainability of this production system would be the use of promoting growth plant rhizobacteria (PGPR). However, care is needed to select a microorganism to be used in crops that are usually consumed raw, so human health is not at risk. It was important to search for PGPR, as rhizobium, that already were broadly used as inoculants for leguminous plants for several decades, without risks to human health. PGPR can promote growth and development of plants through direct and indirect mechanisms, by production and secretion of chemical substances in the rhizosphere. The direct mechanisms were involved with the uptake of nutrients by the plants (nitrogen, phosphorus and essential minerals) through phosphate solubilization, production of siderophores and growth regulators. The indirect mechanisms were involved with the decrease of inhibitory effects from various pathogenic agents related with biological pest control, thereby favoring plant growth. Nevertheless, due to its ability to promote beneficial effects to plants, effective bacterial colonization was extremely important. Some bacteria that colonized the rhizoplane may penetrate the plant roots and some strains may move to the aerial part, with decreased bacterial density, compared with colonizing populations in the rhizosphere or roots. It can be concluded that Rhizobia promotes plant growth using different mechanisms as biological nitrogen fixation and production of different plant growth regulators (e.g. auxins). Therefore, new studies with Rhizobia characterization and observation about its different mechanisms of promoting plant growth should be performed. Such information would be useful for the identification of plants with potential to increase agricultural production due to the benefits of using plant growth promoter's rhizobia.

Key words: Growth promotion, vegetable crops, rhizobacteria, growth regulators.

INTRODUCTION

Rhizobium as plant growth promoters

Bacteria capable of colonizing the rhizosphere or plant roots and also assist direct or indirectly the crop growth and development are called promoting growth plant rhizobacteria (PGPR) (Kloepper et al., 1980; Abbasi et al., 2011). These rhizobacteria have the ability to stimulate crop growth through enrichment of soil nutrients,

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such as biological nitrogen fixation, phosphate solubilization, siderophores production and growth regulators. Furthermore, they may act as a biological control, producing defense substances for the plant, as cellulase, protease, lipase and β-1,3 glucanase (Ahmed et al., 2009; Bulgarelli et al., 2013; Gopalakrishnan et al., 2015).

In the search for a more sustainable agricultural production, the pant-rhizobacteria interactions are important when related to nutrients transformation, mobilization and solubilization, so the plant may absorb nutrients that would otherwise be unavailable for its development (Hayat et al., 2010; Gopalakrishnan et al., 2015). Agricultural production sustainability is based on biological approaches for enhancement of agricultural production, seeking alternatives to reduce fertilizers. Rhizobacteria may be an alternative for assisting cultivars growth and protection.

Several studies are being conducted with the objective of investigating the potential for rhizobacteria to promote plant growth through substances that regulate growth, tolerance of these bacteria to agrochemicals, abiotic stress, among others (Ahamad and Khan, 2012; GurURANI et al., 2013; Gopalakrishnan et al., 2015). However, most of the studies with plant growth promoting bacteria is related to the *Enterobacter*, *Burkholderia*, and *Bacillus* species. Although there are promising results with the use of rhizobium for growth promotion in several crop species as rice, wheat and corn (Hahn, 2013; Osório Filho, 2014), the mechanisms involved in those processes are not yet fully understood. Findings on the use of rhizobium for plant growth broaden the perspectives for its use on diverse agricultural systems and on the different crop species. Initially, when the term rhizobacteria was introduced, it was destined to non-symbiotic bacteria present in the rhizosphere with the ability to colonize the radicular system and favor plant growth (Kloepper and Schroth, 1978).

With the advances of studies on the plant-microbe interactions, new concepts were established. This includes bacteria that establish symbiotic relationships with the plant, in the group of the so called intracellular PGPR (iPGPR), which live inside of the root cells, form nodules and live in specialized structures. These new concepts also include bacteria that develops in the rhizosphere, rhizoplane or intercellular space, without the formation of nodules, but capable of crop growth promotion through the production of specific substances, which is the group called extracellular PGPR (ePGPR) (Gray and Smith, 2005).

Among the intracellular PGPR, the most studied are the rhizobium with leguminous plants symbiosis (Gray and Smith, 2005), and among the extracellular PGPR are the bacteria from the *Bacillus* and *Pseudomonas* genera (HarTEMAN et al., 2010; Rocha and Moura, 2013). There are, however, rhizobacteria that can stablish both in the interior and exterior of cells, belonging to both groups, that is, *Burkholderia* (Gray and Smith, 2005). The best studied example is the beneficial interaction between rhizobium and plants from the Fabaceae family (leguminous), and they are the most used agricultural inoculants in the world. In symbiosis, the rhizobium grows using the carbohydrates supplied by the host plant, and in exchange, provides fixed nitrogen for amino acid biosynthesis (Brencic and Winans, 2005; Gray and Smith, 2005). This symbiosis is an example of the intimal relationship between a soil bacteria and its host plant, and illustrates the concept of iPGPR for soils with nitrogen deficiency. Bacteria convert atmospheric nitrogen (N_2) to ammonia (NH_3), thus promoting leguminous plant growth through the supply of this nutrient (Van Loon, 2007). However, rizhobium may also be classified as ePGPR when associated with non-leguminous plants, being able to accommodate between the spaces among the plant cells or in the rhizosphere, on the roots surface. As such, they stimulate plant growth through several ways, such as: growth regulators production, phosphate solubilization, siderophores production, and more (Moreira et al., 2010; Garcia-Fraile et al., 2012; Flores-Félix et al., 2013).

MECHANISMS OF PLANT GROWTH PROMOTION

Plant-microbe interactions may affect the growth of the crops by direct or indirect mechanisms (Glick, 2012). Among the direct mechanisms, are the biological fixation of nitrogen (BFN); plant growth regulators production such as indolacetic acid (IAA), gibberellins, cytokinins and ethylene; phosphate solubilization; and siderophores production.

Biological nitrogen fixation

Microorganisms capable of atmospheric nitrogen fixation are called diazotrofic and are classified as: symbionts, e.g. rhizobium that establish relationships with leguminous plants or *Frankia* that establishes relationship with non-leguminous plants, free-living, associative and endophytic, as *Azospirillum, Azotobacter* and cyanobacteria (*Anabaena, Nostoc*) (Ahemad and Khan, 2012; Bhattacharyya and Jha, 2012).

Biological fixation of nitrogen (BFN) is the process by which the diazotrofic microorganisms in the soil convert atmospheric N_2 into NH_3, which is the form the plants can assimilate. This process is accomplished through an enzymatic complex called nitrogenase (Zehr et al., 2003; Stefan et al., 2008; Bulgarelli et al., 2013). Through BFN, the nitrogenated compounds are readily available to plants, by associative relations, or released to the environment, by the decomposition of the bacterial biomass (Lindermann and Glover, 2003; Gopalakrishnan et al., 2015).

The symbiotic relationship between rhizobium and

leguminous plants is able to fix approximately 460 kg of nitrogen per year (Bulgarelli et al., 2013). Several studies have demonstrated the importance of BFN by rhizobium, not only because of the benefits for leguminous plants, but also through mixed intercropping, benefiting the species to be planted after the leguminous cultivar (Castro et al., 2004; Hayat et al., 2008). Several free-living bacteria, either associative and/or endophytic, promote the growth of several plants by BFN, that is, bacteria from the *Azospirillum, Burkholderia, Herbaspirillum, Klebsiella, Enterobacter,* and *Citrobacter* genera *(*James et al., 2000; Kennedy et al., 2004; Gholami et al., 2009).

Production of substances that regulate crop growth

Plant growth regulators are chemical compounds that influence and promote the growth and development of plants. The main classes are: auxins, cytokinins, gibberellins, abscisic acid and ethylene (Santner and EstELLE, 2009). Among the auxins, indolacetic acid (IAA) is the most abundant one and it is involved with several cellular processes and processes of crop growth and development. It is also the most physiological active auxin on crops and it is the focus of studies related with promoting growth plant rhizobacteria (Bulgarelli et al., 2013).

Several studies have demonstrated the involvement of rhizobacteria in the biosynthesis of IAA, both in culture and in the soil (Hameed et al., 2004; Khalid et al., 2004; Thakuria et al., 2004). One of the direct effects of IAA producing rhizobacteria is the proliferation of secondary rooting and radicular hairs, enhancing the nutrient absorption by the plants associated with these bacteria and, therefore, improving crop growth and development (Biswas et al., 200; Lambrecht et al., 2000; Machado, 2011).

The ability to produce indolacetic acid is one of the most studied mechanisms in the promotion of plant growth by rhizobacteria, and approximately 80% of bacteria isolated in the rhizosphere can produce IAA. Several studies have demonstrated that most rhizobium is able to produce IAA. It is involved with the processes of cell division and differentiation, which are essential for the nodule formation, when in symbiosis with the leguminous species (Ahemad and Khan, 2012; Hahn, 2013; Osório Filho, 2014; Machado, 2015).

Another growth regulator produced by PGPR are gibberellins, that were named after the compounds excreted from the fungus *Giberella fujikuroi*, which triggered exaggerated growth on rice plant height and suppressed the production of seeds. The first isolated compound from the fungus culture was called gibberellic acid (GA3), which is responsible for stem growth due to the stimuli that the gibberellins promote on cellular elongation rates and division (Taiz and Zeiger, 2013).

On the other hand, gibberellins are never present in tissues with total lack of auxins, and the effects of gibberellins on growth might also depend on the acidification of the environment by auxins. Application of gibberellins is also responsible for parthenocarpy in fruits, by increasing the fruit size and the number of buttons and seed germination, more specifically over the production of α-amylase in the aleurone layer of cereals (Camili, 2007; Taiz and Zeiger, 2013). Gibberellins help in seeds germination, as is the case for lettuce and cereal, and control of flowering and sexual expression of flowers. Many PGPR are described as producing gibberellins (Dobbelaere et al., 2003), including *Rhizobium*, *Sinorhizobiu meliloti* (Boiero et al., 2007).

In the literature, few are the studies about the production of cytokinins by plant growth promoting bacteria. Ortiz-Castro et al. (2008) studied cytokinin signaling in the promotion of crop growth by *Bacillus megaterim*, and found that cytokinin receptors play a complementary role on crop growth promotion. This growth regulator has great capacity to promote cellular division, participating in the process of elongation and cellular differentiation, especially when interacting with auxins (Taiz and Zeiger, 2013).

Ethylene is a growth regulator produced by all plants, and is essential for proper growth and development. When in stressful situations, as drought, salinity, flooding, trace elements, and pathogens, the plant significantly increases production of this growth regulator, triggered by its defense response (Saleem et al., 2007; Bhattacharyya and Jha, 2012). But if the stress persists, the severe increase in ethylene concentration by the plant will trigger senescence processes, chlorosis and abscission, leading to inhibitory effects on growth and development (Stearns and Glick, 2005).

In relation to growth promotion involving ethylene, several studies have been made with bacteria capable to promote plant growth, related to the synthesis of the enzyme ACC (1-aminociclopropano-1-carboxilato) deaminase, which hydrolyses ACC, an immediate precursor of ethylene. Since high concentrations of ethylene act on radicular growth inhibition and senescence, ACC-deaminase regulates the levels of ethylene in order to assist the plant in its growth and development (Onofre-Lemus et al., 2009).

Rhizobacteria capable of producing the enzyme ACC deaminase belong to several genera, as *Bacillus, Burkholderia,* and *Rhizobium*, among others (Zahir et al., 2008; Onofre-Lemus et al., 2009; Kang et al., 2010). These bacteria associated with plants help regulate ethylene levels, acting as ACC sinks, reducing the deleterious effects of ethylene and promoting plant growth. The main effects of plant inoculation with ACC deaminase-producing rhizobacteria are increase in seed germination rates, radicular growth stimulation, enhancement in nutrient absorption, as nitrogen, phosphorus and potassium, and increase in nodulation on

rhizobium *(*Zafar-Ul-Hye et al., 2007; Glick, 2012).

Phosphate solubilization

Phosphorus is the second limiting nutrient for vegetable growth after nitrogen, and is abundant in the soil both on organic and inorganic forms. However, in most soils this element is in low availability to plants, since they can only absorb it in the forms of the soluble ions $H_2PO_4^-$ and HPO_4^{-2} (Khan et al., 2009; Bhattacharyya and Jha, 2012). The search for strategies to enhance the availability of this mineral to plants can significantly improve crop growth and productivity, since the fraction of phosphorus that is available for plants is relatively low in soils (5% of total phosphorus) (Dobbelaere et al., 2003).

The use of microorganisms that solubilize phosphate may assist or substitute the use of phosphate fertilizers in agriculture (Khan et al., 2006). The mechanism used by bacteria to solubilize inorganic phosphorus is through the production of organic acids, while the mineralization of organic phosphorus is through the production of several phosphatases that result in the release of phosphoric acids (Bulgarelli et al., 2013; Glick, 2012). Rhizobacteria as *Rhizobium, Bacillus* and *Pseudomonas*, are efficient in the process of solubilization of inorganic phosphate, making it available for the plants. Studies show the positive effect of inoculation with bacteria that solubilize phosphate on the promotion of plant growth (Marra et al., 2011; Ahemad and Khan, 2012; VikRAM and Hamzehzarghani, 2008).

Production of siderophores

Iron, as well as phosphorus, is an abundant element in soil, but, due to the low solubility of iron oxides, little is available for plants (Rajkumar et al., 2010). Thus, plants need to use strategies to increase Fe availability through either the release of protons in order to reduce the pH of the soil and increase Fe, or the release of a Fe chelating agent, as siderophores, which will bind Fe, so it can be absorbed by the plant roots (Jeong and Guerinot, 2009).

Rhizobacteria, as well as plants, have the ability to produce iron chelating molecules, called siderophores, when there is low availability of Fe for their development. The release of siderophores by the rhizosphere bacteria assists the crop growth, inhibiting the proliferation of pathogens in the roots, due to the competition for Fe in the soil (Dobbelaere et al., 2003; Bulgarelli et al., 2013). Plants have the capacity to absorb the bacterial Fe-siderophores complex, and, once inside the plant, the Fe unbounds from the siderophore and this molecule is then recycled or destroyed (Rajkumar et al., 2010).

In studies with the plant *Arabidopsis thaliana*, it was found the presence of Fe-pyoverdines, a siderophore synthesized by *Pseudomonas fluorescens*, and an increase in Fe content inside the plant and also an increase in crop growth (Vansuyt et al., 2007).

Rhizobacteria also act on biological control by reducing fungus diseases in the plants, and thus promoting plant growth (Dey et al., 2004).

Sequestration and transport of iron on plant cells through siderophores from rhizobium is one of the ways to provide iron to the plants when in conditions of low iron availability in the environment. Several strains of rhizobium can synthesize siderophores, that will bind Fe^{3+}, reduce it to Fe^{2+}, making it available for the plant (Carson et al., 2000; Arora et al., 2001).

INDIRECT MECHANISMS

The main indirect mechanism of crop growth promotion is related to the use of rhizobacteria as agents of biological control against plant pathogens, by induction of resistance and production of antifungal substances. Many PGPR are capable of producing antifungal metabolites, as hydrogen cyanide and enzymes, such as chitinases and glucanases, that degrade the fungal cell wall (Persello-Cartieaux et al., 2003).

The interaction between plants and rhizobacteria stimulates crops to acquire resistance against some pathogenic microorganisms, as bacteria, fungi and viruses. This process is known as induced systemic resistance (ISR), which is caused by the release of some bacterial molecules that activate promoting genes of defensive compounds in the plant (Lugtenberg and Kamilova, 2009).

Strains of *Bacillus*, when used as a biocontrol agent against phytopathogens, use this mechanism of production of antibiotic substances (Kokalisburelle et al., 2006). *P. fluorescens* are bacteria known by the suppression of pathogenic fungi in the soil, by producing antifungal metabolites and releasing siderophores, by making the iron unavailable to the pathogens in the roots (Dwivedi and Johri, 2003). Several species of *Bacillus* and *Pseudomonas* are used in biologic control, such as in tomato plants when inoculated with bacteria from these genera, which leads to a reduction of wither symptoms caused by *Ralstonia solanacearum* and *Fusarium oxysporum f. sp. lycopersici* (Rocha and Moura, 2013).

Besides acting on control of bacteria, fungi and viruses, PGPR may also act on the control of nematodes. In studies with watermelon and melon, inoculation of rhizobacteria decreased nematode attacks to these plants (Kokalis-Burelle et al., 2003). In rice seeds inoculated with PGPR, the control of *Meloidogyne graminicola* associated with growth promotion in plants was found (Souza Junior et al., 2010).

BACTERIAL COLONIZATION

Colonization of plant roots by beneficial rhizobacteria is one important step towards interactions between plant and bacteria. However, it is a complex process that is

influenced by several biotic and abiotic factors, such as quantity of bacteria and root exudation (Benizri et al., 2001). The success of root colonization by rhizobacteria, as its persistence in the rhizosphere, is a fundamental factor for exerting the beneficial effects to the plants. A minimal bacterial density is necessary for the establishment of the molecular, biochemical and physiological mechanisms of plant-microbe interaction, and this concept is called *quorum sensing* (QS) (Williams, 2007; Sanchez-Contreras et al., 2007).

The mechanism of QS depends on synthesis and release of chemical signals by the bacteria in the environment, and detection of these signals, as a function of the cellular population density (Camilli and Bassler, 2006). Such group behavior results in alteration of genetic expression, which drives the activity of the microorganisms in a coordinated manner (Williams, 2007). Among the chemical signs released, the more common used by Gram-negative bacteria are called homoserine lactones, originally called *N*-acyl homoserine lactones (AHLs). Biosynthesis and effects of self-inducers like AHLs rely especially on the activity of a protein family of LuxI and LuxR. After the AHLs are produced by AHL synthases enzymes, they spread across bacterial membranes and accumulate until they reach higher concentrations. In a certain concentration threshold (approximately 10 nM), AHL binds to the gene LuxR, forming a complex that regulates gene expression (Hanzelka and Greenberg, 1995).

Communication via *quorum sensing* by AHLs in rhizobium affects its metabolic and physiologic processes, including mobility, exopolysaccharide synthesis, biofilm formation, production of virulence factors, plasmid transfer, efficiency in root nodulation and efficiency of nitrogen fixation (González and Marketon, 2003; Sanchez-Contreras et al., 2007; Pierson and Pierson, 2007). Studies with rhizobacteria of the genus *Pseudomonas* tagged with fluorescent genes found that, as a consequence of the radicular colonization by these bacteria, there was an increase in the biosynthesis of siderophores, growth regulators, antibiotics and hydrolases (Compant et al., 2010).

CONCLUSION AND RECOMMENDATION

In the search for a more sustainable agricultural production, the interactions between plants and rhizobacteria are important for the plant to absorb nutrients that otherwise would be unavailable for its development. Rhizobium is already used for leguminous plants, with excellent results because of its ability to fix atmospheric nitrogen. They may act as growth promoters in oleraceous plants because they have direct and indirect mechanisms of plant growth promotion. Since most studies involving growth promoting bacteria for oleraceous plants are with the *Pseudomonas* and *Bacillu*s genera, it is important to seek other genera and species.

In the current literature, there are very few studies using rhizobium as growth promoters for oleraceous plants. However, there are various rhizobium species that need to be explored, as well as species and variety of crops, in which the effect of inoculation is poorly studied. Therefore, new studies with Rhizobia characterization and observation about its different mechanisms of promoting plant growth should be performed. Such information would be useful for the identification of plants with potential to increase agricultural production due to the benefits of using plant growth promoter's rhizobia.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of foliar application of iron on seasonal changes of some physical and chemical properties in berries of Halwani Lebanon grape cultivar (*Vitis vinifera* L.)

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A field experiment was conducted in 2009 and 2010 growing seasons, to study the effect of foliar application of iron- chelated "Fe-EDTA" (0, 100 and 200 mg.l^{-1}) on the seasonal dynamic of some physical and chemical properties of berries of Halwani Lebanon grape cultivar, grown on a calcareous soil in Mosul region, Iraq. The results revealed that the foliar application of 100 mgFe.l^{-1} caused a significant increase of berry weight, TSS, glucose and malic acid in berries while spraying with 200 mgFe.l^{-1} caused a significant increase in the total acidity (TA), tartaric acid and fructose in juice berries compared to the control for both seasons. Additionally, berry weight, TSS, glucose and fructose were increased from the beginning of berry growth to véraison and ripening stage in both seasons; the TA, malic acid and tartaric acid increased from berry set to véraison whereas they decreased towards the end of the growth seasons in both seasons. On the other hand, the interaction between iron levels and times on growth and development of berries were also discussed.

Key word: Iron spraying, seasonal changes, grape berry.

INTRODUCTION

Grapes are high in carbohydrates and are useful source of many minerals and vitamins B_6, C, E and K. They are also a source of antioxidant compounds through the phenolic in their skins and possibly seeds (Yilmaz and Toledo, 2004). Halwani Lebanon is considered one of the most important grape cultivars grown successfully in Iraq. Seasonal changes occur in many physical and chemical characteristics of the grapes during growth and development from beery set to maturity. Among micronutrients, iron plays a vital role in synthesis of chlorophyll, carbohydrates production, cell respiration and nitrogen assimilations. In addition to the important function in photosynthesis, it is involved in the biosynthesis of plant hormones (Mengel et al., 2001; Greasy and Greasy, 2009). The protoporphyrin synthesized as a precursor of hem is also a precursor of chlorophyll (Bould et al., 1983). Iron deficiency causes marked changes in the ultrastructure of chloroplast, with thylakoids grana being absent under extreme deficiency and the chloroplast being smaller (Bould et al., 1983; Kirkby and Romheld, 2004). The availability of iron in soil, a function of a number of properties viz., texture, $CaCO_3$

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Table 1. Analysis of the tested soil according to (Page et al., 1982).

Depth (cm)	Clay (%)	Silt (%)	Sand (%)	O.M. (%)	$CaCO_3$ (%)	HCO_3 (meq/L)	Soil pH
0-25	22.3	55.6	22.1	1.36	18.3	2.26	7.63
25-50	19.2	58.4	22.4	0.96	19.6	2.62	7.74
50-75	18.8	59.1	22.1	0.64	19.9	2.69	7.79

Depth (cm)	Available		K (meq/L)	P (ppm)	Total N (%)	CEC (meq/L)	EC (ds/m)
	Zn (ppm)	Fe (ppm)					
0-25	1.00	1.83	0.211	4.0	0.08	23.60	1.20
25-50	1.10	1.70	0.216	3.7	0.08	23.63	1.22
50-75	0.92	1.69	0.214	3.0	0.04	23.50	1.24

content, organic matter, physiological pH range and amount of iron in the soil form which is in equilibrium with these in the soil solution (Lindsay, 1976; Mengel, 1994). The lime induced iron chlorosis is a common problem when crops are grown on calcareous soils. Some of the crops which are sensitive to iron deficiencies are citrus, grapes, vegetable, ornamentals, strawberries and avocado (Tendon, 1998). Furthermore, the presentation of chelated iron increases the possibility of foliar absorption favoring photosynthesis processes.

The objectives of this experiment were to: 1) Investigate the effect of foliar application of Fe-EDTA on berry weight and its some bio-chemicals composition in grape berry juice and to study the effect of seasonal changes (weekly intervals) of berry weight and its some bio-chemical composition of berry growth and development during the growing seasons.

MATERIALS AND METHODS

This experiment was carried out during 2009 - 2010 in the growing seasons on 20-year old vines (*Vitis vinifera* L.) of Halwani Lebanon grapevines cultivar grown at private orchard located at 36.19 N, 43.09 E and at latitude of 222.6 m above mean sea level in the city of Mosul, Nineveh governorate, Iraq. The vines were planted at 2.25 m × 3 m apart. Full description of the tested soil is given in Table 1 according to Page et al. (1982). The vines trained to the cane system were chosen as uniform in vigor. The experimental vines were pruned in mid- February (Al-Imam and Altalib, 1995); left four canes (each with 12 buds) and six spurs (each with 2 buds) per vine. The chosen vines were divided into a different treatment, including the control. Foliar application of Fe-EDTA at three levels (0, 100 and 200 $mg.l^{-1}$) was carried out three times per season; the first time before the start of bloom at April 20, the second time after berry set on May 20, and the third time, 30 days later using Tween-20 as a wetting agent at 0.1% was added to the spraying solution of Halwani Lebanon cultivar. Seasonal changes of the berry weight, total soluble solids (TSS), glucose, fructose, total acidity (TA), tartaric acid and malic acid during the growth phases of berries were studied. Grape berries of Halwani Lebanon cultivar were sampled 14 times separately. The present investigation is a factorial experiment split in time; each treatment was replicated three times with two vines per each and randomized complete block design (RCBD) was arranged. Data obtained throughout this study were statically analyzed using analysis of variance and subjected to Duncan's multiple range test; 0.5 P level was used to differentiate means (Roger and Hasted, 2003).

Grape berries were sampled at 14 weekly intervals from berry set on May 29 throughout fruit ripening on September 1. TSS was determined with hand refract meter; quantity determination of glucose and fructose was done using enthrone (Plummer, 1974). The per cent absorbance was then read at 620 nm by Spectrophotometer with the reagent blank set at zero absorbency. Total acidity was determined against NaOH 0.1 N as tartaric acid (Ranganna, 1986). Quantity determination of tartaric acid was done by the spectrophotometer at 520 nm (Zoecklein et al., 1980) using Sodium meta vanadate material. Quantity determination of Malic acid was done (Jakobs, 1958) by using Calcium acetate.

RESULTS AND DISCUSSION

Berry weight

Data in Figure 1 shows that the berry weight of Halwani Lebanon grape was positively affected in response to foliar application of iron-chelated "Fe-EDTA". The highest value of berry weight was obtained by spraying with 100 $mgFe.l^{-1}$ of iron (3.964 and 4.160 g) compared with the control (3.645 and 3.701 g) in both seasons respectively.

Spraying with iron caused a significant increase in the percentage of pollen vitality, pollen grains germination, length of pollen tube, setting of berries, ovules fertilization and the number of seeds in the berry, in addition to, the increase of chlorophyll content of leaves, and leaf area per cluster used and foliar application of Fe-EDTA at four levels 0, 50, 100 and 200 $mg.l^{-1}$ (Al-Imam, 1998). Increase in photosynthesis sufficiency, and its product is used for cell division and expansion, which has been positively reflected in increasing the berry weight. In general, micronutrient values (Table 1) were under the critical range in calcareous soil of orchard soils of vines. This indicates that the grapevines grown in this orchard might respond to Fe-fertilization, whereas a negative correlation between micronutrients with both pH and carbonate forms appeared. These relations indicated the significant effects of pH and carbonate forms upon the distribution of available micronutrients in calcareous soils (Seddyk et al., 1995). The soils of Nineveh orchards were characterized as calcareous with high $CaCO_3$ content and high pH values that result in decreasing the available

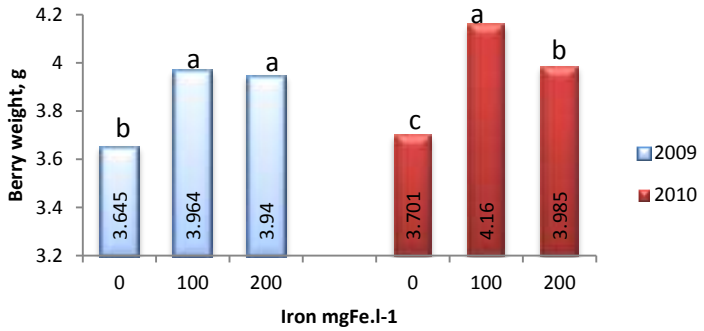

Figure 1. Effect of Fe-EDTA levels on berry weight. Means with the same letter are not significantly different at p=0.05 according to Duncan's test.

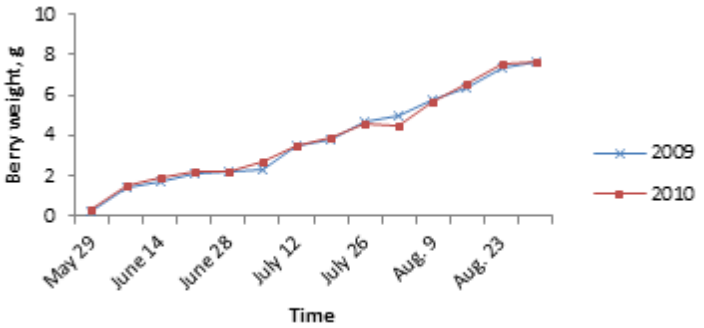

Figure 2. Changes in berry weight measured during the growth and development of Halwani Lebanon.

amount of some nutrients especially micronutrients whose deficiency symptoms appear on grapevines. The results in Figure 2 show that significant changes in grape berries were sampled at 14 weekly intervals and increased dramatically from berry set on May 29 (0.263 and 0.317g) throughout maturity on September 1 (7.603 and 7.640 g) in both seasons respectively.

In the first phase of the berry grape growth, the fertilization results in immediate and rapid cell division in more than approximately 200,000 cells in a thesis to a maximum of 600,000 at berry véraison. It is distinguished by a large change in gene expression (Harris et al., 1968; Davies and Robinson, 2000; Waters et al., 2005). The results of the investigation in Table 2 revealed that the combination of iron levels with sampling dates of berries, had a clear effect on berry weight, especially foliar sprays with 200 and 100 mgFe.l^{-1} of iron and their combinations with a final sampling date of Sept.1 caused a significant increase of berry weight (7.80 and 7.83g) in both seasons respectively. The growth and development of the grapevine berries are usually divided into three major and quite distinct phases. The first-phase shows in Table 2 a period of rapid growth and displays a very active metabolism and rapid cell division, which starts after fruit set on May 29 (0.23g) to June 21 (2.00g). In these 4 weeks there was a significant increase of berry weight. In the second phase-period of about 3 weeks there was a slow and slight increase in berry weight from June 21 (2.00 g) to July 12 (2.21g) at véraison. The French word véraison used to describe the change in berry skin color (Conde et al., 2007) indicates the beginning of ripening. After véraison, berries resume fast growth again from July 19 (3.48 and 3.51 g) to fruit maturity on Sept.1 (7.20 and 7.31 g) of berry weight in both seasons respectively. The berries start to accumulate water and carbohydrates especially sugars and other color levels bred to increase fruit size and weight. The most dramatic changes in the grape berry composition occur during this ripening phase (Winkler et al., 1974; Monselise and Raton, 1986; Conde et al., 2007). In addition to the development of fruit tissue represents the final phase of floral development and involves both cell division and cell expansion (O'Neill, 1997).

Foliar sprays is most effective, when soil nutrient availability is low (Table 1), topsoil dry and root activity during the reproductive stage is decreased (Wojcik, 2004). Data of soil analysis listed in Table 1 revealed that the soil of the experimental orchard contained high pH, percentage of $CaCO_3$ and low organic matter. The predominantly calcareous of high pH soils could limit the availability of micronutrient, including Fe, Mn, Cu and Zn, since they tend to precipitate in soil solution in a

Table 2. Interaction effect of Fe-EDTA and seasonal times on berry weight of cv. Halwani Lebanon grape.

Iron (mg.l^{-1})	2009			2010		
	0	100	200	0	100	200
May 29	0.23^{t}	0.33^{t}	0.23^{t}	0.27^{n}	0.40^{n}	0.28^{N}
June 7	1.01^{s}	1.58^{r}	1.63^{qr}	1.03^{m}	1.88^{l}	1.68^{L}
June 14	1.68^{p-r}	1.74^{o-r}	1.86^{n-r}	1.90^{l}	1.93^{l}	1.87^{L}
June 21	2.00^{n-r}	2.13^{n-q}	2.10^{n-q}	2.27^{l}	2.30^{l}	2.08^{L}
June 28	2.07^{n-r}	2.24^{no}	2.18^{n-p}	2.30^{l}	2.40^{l}	1.98^{L}
July 5	2.21^{no}	2.35^{n}	0.22^{no}	2.36^{l}	3.45^{jk}	2.18^{L}
July 12	3.18^{m}	3.53^{lm}	3.66^{k-m}	3.15^{k}	3.75^{i-k}	3.71^{i-k}
July 19	3.48^{lm}	3.73^{kl}	4.01^{k}	3.51^{jk}	3.81^{i-k}	4.27^{g-i}
July 26	4.55^{j}	4.72^{ij}	4.87^{j}	4.10^{h-j}	4.83^{fg}	4.91^{e-g}
Aug. 2	4.72^{ij}	5.00^{h-j}	5.11^{hi}	4.73^{f-h}	5.06^{ef}	5.08^{Ef}
Aug. 9	5.42^{gh}	6.03^{ef}	5.81fg	5.41^{ef}	6.10^{cd}	5.58^{De}
Aug. 16	6.12^{ef}	6.60^{d}	6.41^{de}	6.25^{c}	6.71^{bc}	6.69^{Bc}
Aug. 23	7.17^{c}	7.70^{ab}	7.27^{bc}	7.22^{ab}	7.78^{a}	7.70^{A}
Sept. 1	7.20	7.80^{a}	7.81a	7.31^{ab}	7.83^{a}	7.78^{A}

Means with the same letter are not significantly different at p=0.05 according to Duncan's test.

Table 2. Interaction effect of Fe-EDTA and seasonal times on berry weight of cv. Halwani Lebanon grape.

Iron (mg.l^{-1})	2009			2010		
	0	100	200	0	100	200
May 29	0.23^{t}	0.33^{t}	0.23^{t}	0.27^{n}	0.40^{n}	0.28^{N}
June 7	1.01^{s}	1.58^{r}	1.63^{qr}	1.03^{m}	1.88^{l}	1.68^{L}
June 14	1.68^{p-r}	1.74^{o-r}	1.86^{n-r}	1.90^{l}	1.93^{l}	1.87^{L}
June 21	2.00^{n-r}	2.13^{n-q}	2.10^{n-q}	2.27^{l}	2.30^{l}	2.08^{L}
June 28	2.07^{n-r}	2.24^{no}	2.18^{n-p}	2.30^{l}	2.40^{l}	1.98^{L}
July 5	2.21^{no}	2.35^{n}	0.22^{no}	2.36^{l}	3.45^{jk}	2.18^{L}
July 12	3.18^{m}	3.53^{lm}	3.66^{k-m}	3.15^{k}	3.75^{i-k}	3.71^{i-k}
July 19	3.48^{lm}	3.73^{kl}	4.01^{k}	3.51^{jk}	3.81^{i-k}	4.27^{g-i}
July 26	4.55^{j}	4.72^{ij}	4.87^{j}	4.10^{h-j}	4.83^{fg}	4.91^{e-g}
Aug. 2	4.72^{ij}	5.00^{h-j}	5.11^{hi}	4.73^{f-h}	5.06^{ef}	5.08^{Ef}
Aug. 9	5.42^{gh}	6.03^{ef}	5.81fg	5.41^{ef}	6.10^{cd}	5.58^{De}
Aug. 16	6.12^{ef}	6.60^{d}	6.41^{de}	6.25^{c}	6.71^{bc}	6.69^{Bc}
Aug. 23	7.17^{c}	7.70^{ab}	7.27^{bc}	7.22^{ab}	7.78^{a}	7.70^{A}
Sept. 1	7.20	7.80^{a}	7.81a	7.31^{ab}	7.83^{a}	7.78^{A}

Means with the same letter are not significantly different at p=0.05 according to Duncan's test.

carbonate-dominated environment (Epstein and Bloom, 2005). Foliar fertilization might be due to the beneficial effect of iron increase, iron availability and quicker direct uptake of ferrous iron (Fe-EDTA) by vine leaves resulting in better absorption and translocation of N, P, K, Fe and Zn (Al-Imam, 2014). These mineral statues affected the physiological performances of photosynthesis activity and its products and ultimately fruit quality.

Total soluble solids (TSS)

Figure 3 shows that foliar application of 100 mgFe.l^{-1} of iron caused a significant increase in TSS of berry juice (8.464 and 8.121%) compared to 200 and 0 mgFe.l^{-1} of iron in both seasons respectively. From the results shown foliar spraying especially with high level of iron-chelated caused (at 200 mgFe.l^{-1}) a significant decrease of sugar content in the berries, because there is an inverse relationship between the grapevine yield and the number of clusters and the decrease of sugar content in addition to increase tartaric and malic acids in berries (Bravdo et al., 1985; Al-Imam, 1998). The sugars of the vinifera grape are primarily glucose and fructose, generally accounting for 90% or more of the carbohydrates in the must and from 12 to 27% or more of the weight of the

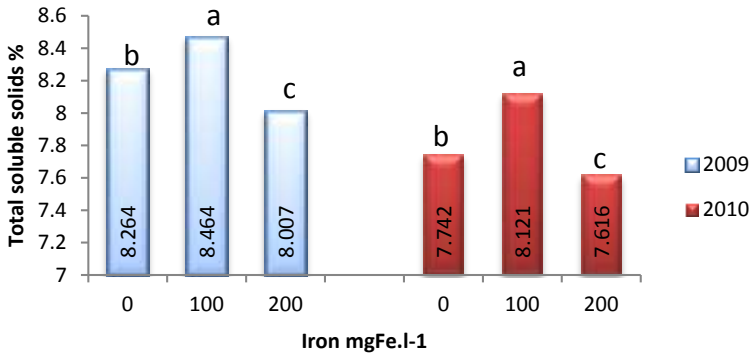

Figure 3. Effect of Fe-EDTA levels on Total Soluble Solids percentage. Means with the same letter are not significantly different at p=0.05 according to Duncan's test.

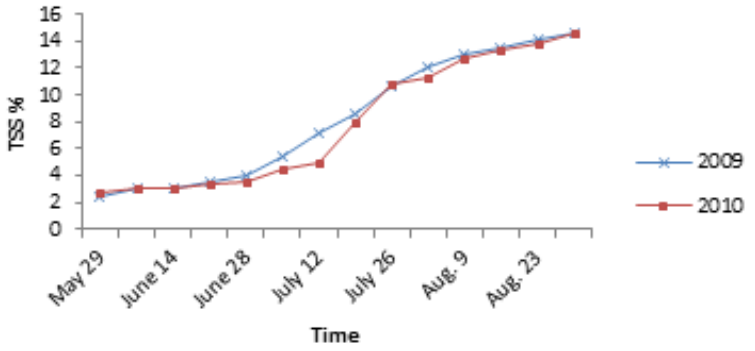

Figure 4. Changes in Total Soluble Solids percentage during the growth and development of cv. Halwani Lebanon grape berries.

mature berry. The trend lines of the berries were sampled at 14 weekly intervals from berry set on May 29 through fruit ripening on Sept.1 of TSS were presented in graphic form in Figure 4 in the berry juice.

There was a slow rise in TSS from May 29 samples (2.5 and 2.667%) to July 5 samples (4.033 and 3.478%) in both seasons respectively. After July 5 the increase in TSS was very rapid in berries and this trend continued up to maturity (14.633 and 14.567%) which were significantly superior to all sample dates in both seasons respectively. The analysis of variance of TSS (Table 3) showed highly significant iron levels × sampling dates interaction. During the first period of rapid growth of the berries the percentage of sugar present is low. During the second stage of growth and development of berries the sugars increase rapidly. During early summer the vines are growing rapidly, most of the sugars are then being used in the growth of the vine and in the increase of berry weight and size. The carbohydrate (Sugar and Starch) that begins to accumulate in the leaves and woody parts of the vine are translocated to the fruits, where there is a rapid buildup of sugars. Another possible source of the sugars in grape berries is from transformation of organic acids from malic and tartaric acids (Winkler et al., 1974; Conde et al., 2007; Greasy and Greasy, 2009).

The summer season in Iraq is hot and the heat summation is rapid, the grapes ripen faster. The amount of TSS content of berries at different iron levels significantly increased during the berry growth season. A significantly greater content of TSS in berries was recorded on September 1 collected from spraying the vines with 0 mgFe.l-1 of iron (15.10%) in the first season and from the spraying the vines with 0 and 200 mgFe.l-1 of iron (14.90 and 14.90%) in the second season respectively; while the lowest amount of TSS was recorded in May 29 from all the three iron levels.

Glucose and fructose in berry juice

The major carbohydrate compounds of the grape berry are glucose and fructose. During ripening glucose and fructose accumulate in roughly equal amounts in the vacuole (Agaeorges et al., 2000). The results in Figure 5

Table 3. Interaction effect of Fe-EDTA and seasonal times on total soluble solids (TSS) percentage in berry juice of cv. Halwani Lebanon grape.

Iron (mg.l^{-1})	2009			2010		
	0	100	200	0	100	200
May 29	*2.50*^{w}	2.40^{w}	2.60^{vw}	2.60^{uv}	2.50^{V}	2.90^{t-v}
June 7	3.10^{tu}	3.00^{uv}	3.00^{uv}	3.20st	3.00^{Tu}	3.00^{tu}
June 14	3.20^{s-u}	3.10^{tu}	3.10^{tu}	3.10st	3.20St	3.10st
June 21	3.50^{r-t}	3.60^{rs}	3.50^{r-t}	3.20st	3.50^{Ro}	3.20st
June 28	3.90^{qr}	4.30^{q}	3.90^{qr}	3.50^{rs}	3.70^{R}	3.23st
July 5	5.40^{p}	5.50^{p}	5.30^{P}	4.30^{q}	4.50^{Pq}	4.50^{pq}
July 12	7.50^{n}	7.00^{o}	7.00^{O}	4.80^{p}	5.50^{O}	4.80^{p}
July 19	8.00^{m}	9.80^{l}	8.10^{M}	7.80^{n}	8.10^{N}	7.90^{n}
July 26	10.50^{k}	11.50^{j}	10.00^{L}	10.50^{m}	11.50^{K}	10.50^{mn}
Aug. 2	12.10^{i}	12.00^{i}	11.90^{lj}	11.00^{l}	11.90^{Jk}	11.00^{l}
Aug. 9	13.00^{gh}	13.60^{ef}	12.70^{H}	12.40^{i}	13.50^{e-g}	12.10^{ij}
Aug. 16	13.50^{ef}	13.70^{de}	13.20^{fg}	13.20^{gh}	13.80^{d-f}	12.90^{h}
Aug. 23	14.40^{be}	14.30^{bc}	13.70^{de}	13.90^{c-e}	14.30^{Bc}	13.40^{op}
Sept. 1	15.10^{a}	14.70^{ab}	14.10^{cd}	14.90^{a}	14.70^{Ab}	14.90^{a}

Means with the same letter are not significantly different at p=0.05 according to Duncan's test.

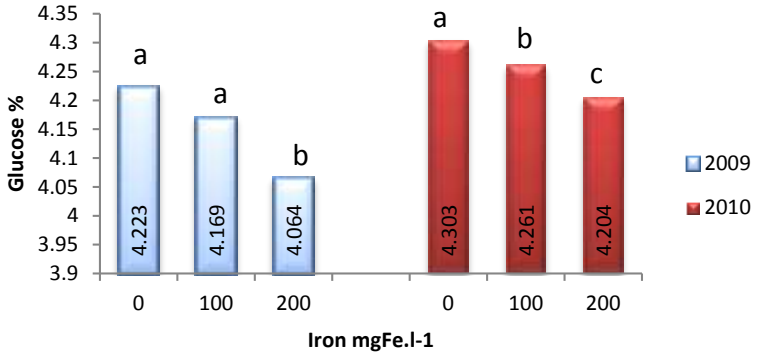

Figure 5. Effect of Fe-EDTA levels on Glucose percentage. Means with the same letter are not significantly different at p=0.05 according to Duncan's test.

showed that spraying the grapevines with 200 mgFe.l^{-1} caused a significant decrease in the glucose content of berry juice as compared with 0 mgFe.l^{-1} in both seasons while in Figure 6 it showed a significant increase of fructose content by increasing the level of iron in the spaying solution especially in the first season.

Spraying with 200 mgFe.l^{-1} caused a highest amount of fructose (3.284 and 3.195%) in the both season respectively. Figures 7 and 8 clearly show that the seasonal changes in glucose and fructose percentage in berries by the time from berry set May 29 to maturity in September 1. Figures 7 and 8 clearly show that the concentration of glucose was higher than fructose from May 29 to July 5 collected and glucose amount significantly increased gradually to maturity. While, the fructose amount increased rapidly after August 9 to maturity (Figure 8). The ratio of glucose to fructose in the grape changes considerably between fruit set until fruit maturity.

The analysis of variance of glucose and fructose (Tables 4 and 5) showed the effect of interaction of iron levels × sampling dates. It was shown that the highest value of glucose (Table 4) shown at the vines sprayed with 0 and 100 mgFe.l^{-1} (7.87 and 7.70%) respectively in the first season and at 0 mgFe.l^{-1} of iron (7.93%) in the second season, were significantly superior to other treatments. While the data in Table 5 clearly shows that the highest amount of fructose at foliar application of 200 mgFe.l^{-1} on Sept.1 at maturity (7.31 and 7.28%) in both seasons respectively, were significantly superior to other

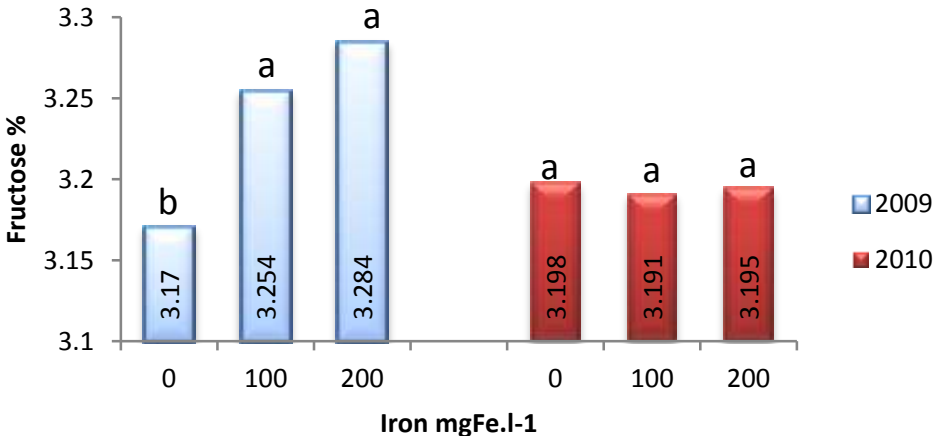

Figure 6. Effect of Fe-EDTA levels on fructose percentage. Means with the same letter are not significantly different at p=0.05 according to Duncan's test.

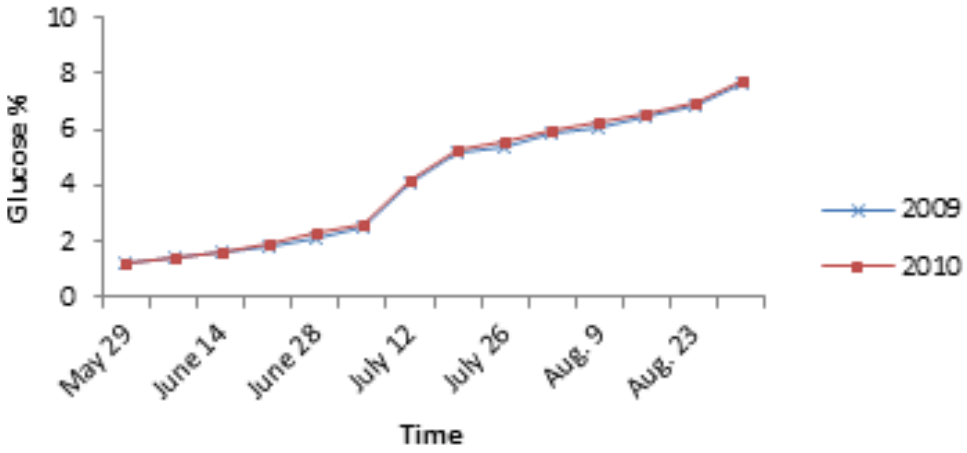

Figure 7. Changes in glucose percentage during the growth and development of cv. Halwani Lebanon grape berries.

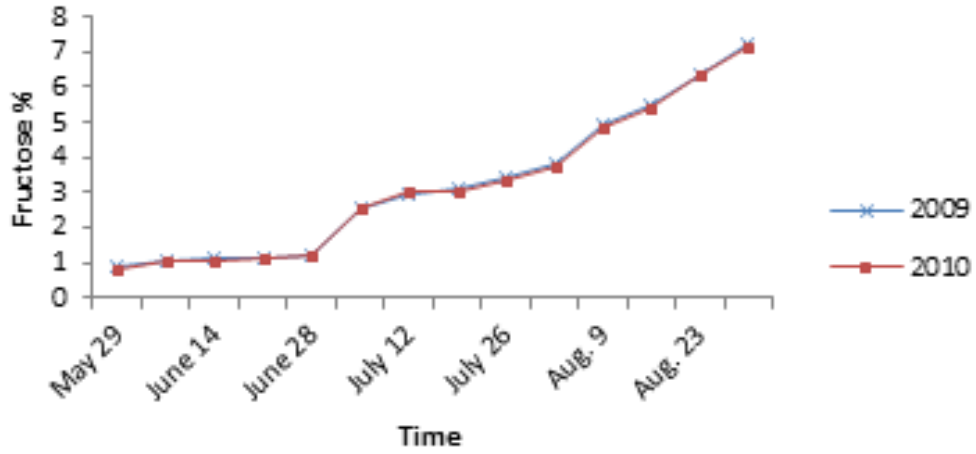

Figure 8. Changes in Fructose percentage during the growth and development of cv. Halwani Lebanon grape berries.

Table 4. Interaction effect of Fe-EDTA and seasonal times on Glucose percentage in berry juice of cv. Halwani Lebanon grape.

Iron (mg.l^{-1})	2009			2010		
	0	100	200	0	100	200
May 29	1.27^{rs}	1.22^{s}	1.33^{s}	1.28^{w}	1.24^{w}	1.28^{w}
June 7	1.42^{q-s}	1.40^{q-s}	1.39^{q-s}	1.44^{v}	1.49^{v}	1.40^{v}
June 14	1.59^{pq}	1.55^{p-r}	1.56^{pq}	1.67^{u}	1.60^{u}	1.59^{u}
June 21	1.80^{p}	1.83^{p}	1.81^{p}	1.90^{t}	1.91^{t}	1.87^{t}
June 28	2.1^{o}	2.20^{no}	2.10^{o}	2.19^{s}	2.39^{r}	2.20^{s}
July 5	2.5^{m}	2.60^{m}	2.40^{mn}	2.61^{q}	2.68^{q}	2.45^{r}
July 12	4.35^{k}	4.0^{l}	4.03^{l}	4.40^{o}	4.11^{p}	4.10^{p}
July 19	5.19^{ij}	5.21^{ij}	5.0^{j}	5.30^{n}	5.33^{mn}	5.31^{n}
July 26	5.28^{i}	5.41^{i}	5.33^{i}	5.41^{m}	5.60^{l}	5.75^{k}
Aug. 2	5.93^{gh}	5.87^{h}	5.80^{h}	5.99^{j}	5.91^{j}	5.91^{j}
Aug. 9	6.23^{f}	6.17^{fg}	5.77^{h}	6.31^{h}	6.21^{i}	6.21^{i}
Aug. 16	6.61^{de}	6.40^{ef}	6.31^{f}	6.70^{f}	6.48^{g}	5.91^{j}
Aug. 23	6.98^{c}	6.80^{cd}	6.74^{cd}	7.11^{d}	6.91^{e}	6.89^{e}
Sept. 1	7.87^{a}	7.70^{a}	7.43^{b}	7.93^{a}	7.80^{b}	7.5^{c}

Means with the same letter are not significantly different at p=0.05 according to Duncan's test.

Table 5. Interaction effect of Fe-EDTA and seasonal times of Fructose percentage in berry juice of cv. Halwani Lebanon grape.

Iron (mg.l^{-1})	2009			2010		
	0	100	200	0	100	200
May 29	0.85^{j}	0.87^{j}	0.88^{j}	0.80^{m}	0.81^{m}	0.83^{m}
June 7	1.07^{ij}	1.10^{ij}	1.10^{ij}	1.00^{l}	1.08^{l}	1.09^{l}
June 14	1.08^{ij}	1.11^{ij}	1.11^{ij}	1.00^{l}	1.09^{l}	1.09^{l}
June 21	1.08^{ij}	1.11^{ij}	1.11^{ij}	1.09^{l}	1.10^{l}	1.10^{l}
June 28	1.21^{i}	1.24^{i}	1.26^{i}	1.18^{l}	1.20^{l}	1.20^{l}
July 5	2.50^{h}	2.00^{h}	2.63^{h}	2.51^{k}	2.50^{k}	2.58^{k}
July 12	2.91^{g}	3.00^{g}	3.02^{g}	3.23^{gh}	2.91^{j}	2.95^{ij}
July 19	3.11^{g}	3.16^{g}	3.17^{g}	3.10^{hi}	3.00^{ij}	3.00^{ij}
Aug. 2	3.78^{e}	3.83^{e}	3.87^{e}	3.80^{f}	3.80^{f}	3.71^{f}
Aug. 9	4.88^{d}	4.95^{d}	4.99^{d}	4.91^{e}	4.90^{e}	4.80^{e}
Aug. 16	5.33^{c}	5.50^{c}	5.58^{c}	5.38^{d}	5.41^{d}	5.40^{d}
Aug. 23	6.28^{b}	6.31^{b}	6.45^{b}	6.30^{c}	6.37^{c}	6.33^{c}
Sept. 1	7.10^{a}	7.31^{a}	7.31^{a}	7.09^{b}	7.11^{b}	7.28^{a}

Means with the same letter are not significantly different at p=0.05 according to Duncan's test.

combination treatments.

Total acidity (TA) in berry juice

Figure 9 clearly shows that spraying with 200 mgFe.l^{-1} of iron chelated caused a significant increase in total acidity (TA) as tartaric acid with the increase of iron concentration in the spraying solution in both seasons.

The total acidity of berries of Halwani Lebanon grape increased fast from berry set on May 29 to June 21 in the first season, and on June 14 in the second season. After these sampling dates the TA decreased slowly until July 19. After that, there was a sharp decrease in TA until Sept.1 at maturity in both seasons (Figure 10).

Tartaric acid was synthesized most rapidly by young developing leaves and immature fruits in the first phase of berry growth. These explain the higher amounts of total acidity found early in the seasons in immature fruits (Kliewer and Lider, 1968). Malic acid is rapidly lost during

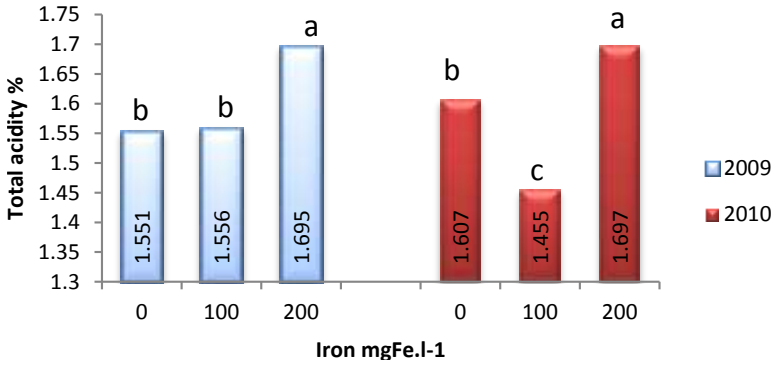

Figure 9. Effect of Fe-EDTA levels on total acidity percentage. Means with the same letter are not significantly different at p=0.05 according to Duncan's test.

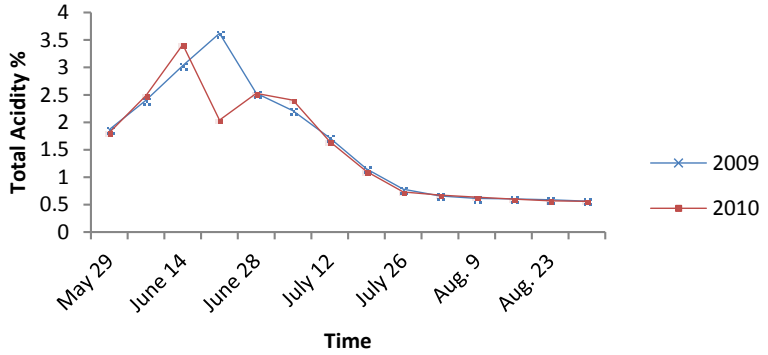

Figure 10. Changes in Total Acidity percentage during the growth and development of cv. Halwani Lebanon grape berries.

warm temperatures and during ripening, while tartaric acid salts are more stable. The decrease in acid concentration is due to an increase in membrane permeability allowing more acid to be metabolized. A reduction in the amount of acids translocated from the leaves, and the formation of salts, mainly potassium salts. A reduced synthesis of acid, the berries, finally, has a dilution effect, due to the rapid increase in berry volume during ripening (Monselise and Raton, 1986). The changes in total acidity in grape berries were evident from means of iron levels × sampling dates interaction (Table 6) and there was a significant increase in TA for 200 mgFe.l^{-1} on June 21 (3.83 and 4.13%) for both seasons respectively. After these sampling dates the TA decreased through Sept.1 at maturity.

Tartaric acid in berry juice

The results over two seasons indicated that foliar application with 200 mgFe.l^{-1} of iron showed a significant increase in tartaric acid in berry juice as compared with the 0 and 100 mgFe.l^{-1} of iron treatments (Figure 11). Seasonal changes of tartaric acid were found in Figure 12 to increase up to the 6th week of berry development during green berry stage to véraison in both seasons.

The highest amount of tartaric acid obtained at the véraison July 12 of berry development was 1.00 and 0.869% in both seasons. After July 12 there was a continuous decrease in the rate of tartaric acid till maturity on Sept.1 in both seasons. The analysis of variance between iron levels × sampling dates was evident from means of the combination in Table 7 and there were significant differences between iron levels in tartaric acid for all sampling dates; especially, the berries sampled of July 12 (1.11 and 0.93%) which were sprayed with 200 mgFe.l^{-1} of iron had a significantly greater amount of tartaric acid in both seasons respectively. The lower amount of tartaric acid (0.29%) was recorded on Sept.1 which was sprayed with 0 mgFe.l^{-1} (control) for both seasons.

Malic acid in berry juice

The data in Figure 13 indicated that spraying with 100

Table 6. Interaction effect of Fe-EDTA and seasonal times of Total Acidity (TA) percentage in berry juice of cv. Halwani Lebanon grape.

Iron ($mg.l^{-1}$)	2009			2010		
	0	100	200	0	100	200
May 29	2.00^{h}	1.71^{lj}	1.89^{hi}	1.95^{i}	1.73^{j}	1.75^{lj}
June 7	2.53^{c-f}	2.56^{c-e}	2.34^{fg}	2.51^{e-g}	2.55^{e-g}	2.40^{f-h}
June 14	2.60^{cd}	3.17^{B}	3.33^{B}	3.65^{b}	3.15^{d}	3.45^{C}
June 21	3.29^{b}	3.75^{A}	3.83A	2.59^{ef}	2.40^{f-h}	4.13^{A}
June 28	2.48^{d-f}	3.40^{e-g}	2.71^{C}	2.50^{e-g}	2.38^{f-h}	2.70^{E}
July 5	2.27^{g}	1.71^{lj}	2.65^{cd}	2.33^{gh}	2.25^{h}	2.63E
July 12	1.75^{i}j	1.69^{J}	1.71^{ij}	1.84^{ij}	1.31^{k}	1.80^{lj}
July 19	1.14^{l}	0.98^{Lm}	1.33^{K}	1.43^{k}	0.90l^{m}	0.98^{L}
July 26	0.72^{n-p}	0.78^{No}	0.84^{mn}	0.74^{mn}	0.70^{n}	0.75^{Mn}
Aug. 2	0.63^{op}	0.67^{n-p}	0.68^{n-p}	0.66^{n}	0.67^{n}	0.69^{Mn}
Aug. 9	0.60^{op}	0.62^{Op}	0.62^{op}	0.61^{n}	0.64^{n}	0.66^{N}
Aug. 16	0.59^{op}	0.61^{Op}	0.61^{op}	0.58^{n}	0.60^{n}	0.62^{N}
Aug. 23	0.57^{op}	0.59^{Op}	0.60^{op}	0.56^{n}	0.55^{n}	0.60^{N}
Sept. 1	0.54^{p}	0.56^{P}	0.59^{op}	0.55^{n}	0.54^{n}	0.59^{N}

Means with the same letter are not significantly different at p=0.05 according to Duncan's test.

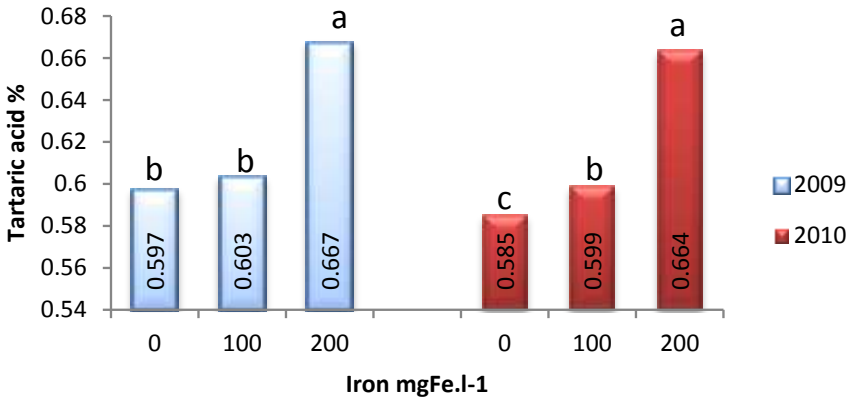

Figure 11. Effect of Fe-EDTA levels on Tartaric acid. Means with the same letter are not significantly different at p=0.05 according to Dancan's test.

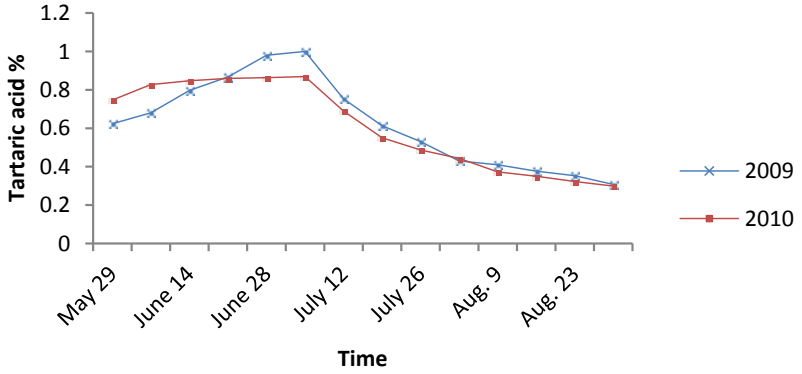

Figure 12. Changes in Tartaric acid percentage during the growth and development of cv. Halwani Lebanon grape berries.

Table 7. Interaction effect of Fe-EDTA and seasonal times of Tartaric acid percentage in berry juice of cv. Halwani Lebanon grape.

Iron (mg.l^{-1})	2009			2010		
	0	100	200	0	100	200
May 29	0.61l^{m}	0.62^{k-m}	0.64^{j-l}	0.70^{ij}	0.74^{g-i}	0.80^{f}
June 7	0.70^{g-i}	0.66^{i-k}	0.68^{h-j}	0.75^{gh}	0.85^{e}	0.88^{b-e}
June 14	0.78^{f}	0.80^{Ef}	0.81^{ef}	0.78^{fg}	0.86^{de}	0.90^{a-d}
June 21	0.88^{cd}	0.84^{De}	0.88^{cd}	0.80^{f}	0.87^{c-e}	0.91^{a-c}
June 28	0.89^{c}	0.97^{B}	1.08^{a}	0.80^{f}	0.87^{c-e}	0.92^{ab}
July 5	0.90^{c}	0.99^{B}	1.11^{a}	0.79^{f}	0.88^{b-e}	0.93^{a}
July 12	0.73^{g}	0.63^{Kl}	0.90^{c}	0.73^{h-j}	0.65^{kl}	0.69^{jk}
July 19	0.60^{lm}	0.53^{N}	0.71^{gh}	0.62^{l}	0.65^{kl}	0.55^{m}
July 26	0.51^{n}	0.50^{N}	0.58^{m}	0.51^{mn}	0.43^{o}	0.52^{mn}
Aug. 2	0.40^{p-r}	0.44^{Op}	0.45^{o}	0.42^{o}	0.41^{op}	0.50^{n}
Aug. 9	0.38^{rs}	0.42^{o-r}	0.43^{o-q}	0.35^{qr}	0.37^{pq}	0.40^{op}
Aug. 16	0.36^{s-u}	0.38^{Rs}	0.39^{q-s}	0.33^{q-t}	0.35^{qr}	0.37^{pq}
Aug. 23	0.33^{t-v}	0.36^{s-u}	0.37st	0.31^{r-t}	0.32^{r-t}	0.34^{q-s}
Sept. 1	0.29^{v}	0.31^{V}	0.32^{uv}	0.29^{t}	0.30st	0.31^{r-t}

Means with the same letter are not significantly different at p=0.05 according to Duncan's test.

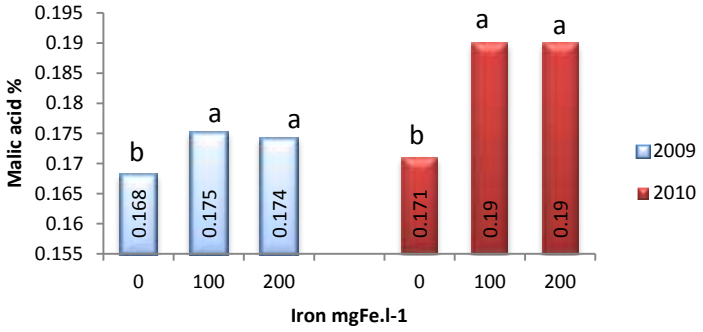

Figure 13. Effect of Fe-EDTA levels on Malic acid. Means with the same letter are not significantly different at p=0.05 according to Dancan's test.

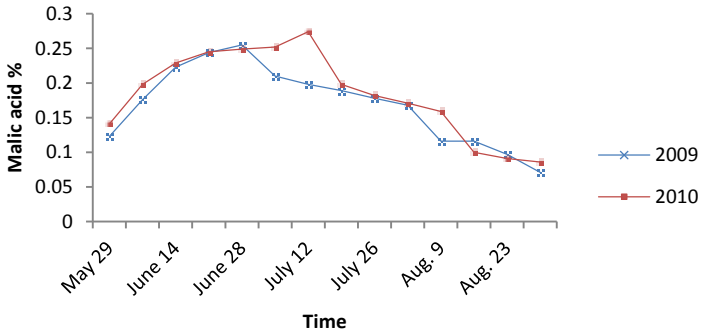

Figure14. Changes in Malic acid percentage during the growth and development of cv. Halwani Lebanon grape berries.

and 200 mgFe.l^{-1} of iron significantly increased the malic acid in berry juice compared to 0 mgFe.l^{-1} of iron treatments. Figure 14 clearly showed that malic acid gradually increased until July 5 (0.255%) in the first season and July 19 in the second season, and then started to decrease at maturity. Malic acid decreases

Table 8. Interaction effect of Fe-EDTA and seasonal times of Malic acid percentage in berry juice of cv. Halwani Lebanon grape.

Iron (mg.l^{-1})	2009			2010		
	0	100	200	0	100	200
May 29	0.129^{o}	0.12^{op}	0.121op	0.13^{n}	0.145^{m}	0.147^{M}
June 7	0.17^{l-n}	0.18^{i-l}	0.177^{j-m}	0.16^{lm}	0.215^{hi}	0.22^{Hi}
June 14	0.20^{fg}	0.224^{cd}	0.246^{b}	0.205^{i}	0.24^{d-f}	0.242^{c-f}
June 21	0.233^{c}	0.249^{ab}	0.251^{ab}	0.230^{f-g}	0.25^{b-e}	0.255^{b-d}
June 28	0.25^{ab}	0.25^{ab}	0.26^{a}	0.236^{e-g}	0.255^{b-d}	0.256^{b-d}
July 5	0.22^{de}	0.254^{ab}	0.201^{fg}	0.24^{d-f}	0.258^{bc}	0.258^{bc}
July 12	0.196^{gh}	0.21^{ef}	0.199^{fg}	0.265^{ab}	0.278^{a}	0.28^{a}
July 19	0.185^{h-k}	0.199f^{g}	0.192^{g-i}	0.185^{jk}	0.221^{gh}	0.188^{j}
July 26	0.173^{k-m}	0.19g^{-j}	0.182^{i-l}	0.18^{jk}	0.182^{jk}	0.183^{jk}
Aug. 2	0.164^{mn}	0.169^{l-n}	0.172^{k-m}	0.17^{kl}	0.171^{kl}	0.172^{j-l}
Aug. 9	0.157^{n}	0.163^{mn}	0.164^{mn}	0.16^{lm}	0.16^{lm}	0.160^{lm}
Aug. 16	0.113^{p}	0.117^{op}	0.117^{op}	0.100^{op}	0.100^{op}	0.11^{o}
Aug. 23	0.093^{q}	0.100^{q}	0.100^{q}	0.095^{op}	0.095^{op}	0.100^{op}
Sept. 1	0.07^{r}	0.090^{q}	0.054^{s}	0.091^{pq}	0.091^{pq}	0.095^{op}

Means with the same letter are not significantly different at p=0.05 according to Duncan's test.

more rapidly than tartaric acid.

The analysis of variance (Table 8) showed a significant interaction between iron levels × sampling dates for data on malic acid in berry juice. The highest value of malic acid is shown in the vines sprayed with 200 mgFe.l^{-1} of iron in June 28 in the first season. While the highest amount of malic acid was obtained on the vines sprayed with 200 mgFe.l^{-1} of iron on July 12 at véraison in the second season. The lower amount of malic acid in the berry juice was obtained at maturity on Sept.1 and sprayed with 200 mgFe.l^{-1} of iron (0.054%) in the first season and in the vines sprayed with 0 or 100 mgFe.l^{-1} of iron in the second season. Grape berries are characterized by large amount of tartaric acid together with malic acid. The two organic acid accounts for more than 90% of the total acidity of the grape berry (Monselise and Raton, 1986).

Conclusion

Foliar application of iron level increased grapevine berry weight, TSS, TA, tartaric acid, malic acid and fructose in berry juice. Berry weight and its bio-chemical products were changed according to the physiological seasonal growing stage. There was high increase in berry weight, TSS, glucose and fructose from berry set to fruit maturity. High concentrations of total acidity, tartaric acid and malic acid to vréraison decreased in mature berries stage especially at ripening.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Seed germination of seeds preserved for about 20 years and that of seeds preserved for several years

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From the herbage seeds, only alfalfa (*Medicago sativa*) showed about 3 - 5% germination ratio for 20 years old seeds. The results are consistent with that of Park and Kim (2009). No germination was observed from the other herbage species, but the decay of seeds were observed sometimes. However, the vegetable seeds preserved for several years showed high germination ratios. In conclusion, older seeds generally germinate a little, while alfalfa seeds continue to live for long time. In addition, new seeds generally germinate more, while, for example, young summer radish germinated a little.

Key words: Seed germination, *Medicago sativa,* genetic diversity, herbage seeds.

INTRODUCTION

There are several reports about genetic diversity, germplasm or seed of plant (Admas and Tesfaye, 2017; Ali et al., 2018; Arantes et al., 2018; Chalachew et al., 2017; Chandra et al., 2017; Daniel et al., 2017; Dilooshi et al., 2016; Santos et al., 2017; Ejigu et al., 2018; Emmanuel et al., 2018; Isaac et al., 2016; Lopez-Puc and Rodriguez-Buenfil, 2017; Revolti et al., 2018; Miccah et al., 2016; Mohammad et al., 2016; Mounawer et al., 2016; Mulima et al., 2018; Ochieng et al., 2015; Pacôme et al., 2016; Raimundo et al., 2015; Rodrigues et al., 2016; Tadesse et al., 2018; Titilayo et al., 2018; Zerihun et al., 2018), fungi (Edelvio, 2018; Ling et al., 2016; Shubha and Srinivas, 2017), bacteria (Chaohe et al., 2015; Paul et al., 2016), and virus (Chikoti et al., 2015).

Investigation was carried on germination and growth of old alfalfa (*Medicago sativa* L.) seeds on soil (2009), seedling growth of some forages from their aged seeds (2012), survey on seed decay during their germination of some forages from their aged seeds' (2013). Recently, study was conducted on another germination test both with unaged vegetable seeds and with the old herbage (or forage) seeds. The purpose of this experiment was to know the viability of more advanced and older herbage (or forage) seeds compared to previous experiments, and

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Table 1. Herbage seeds preserved for about 20 years and vegetable seeds preserved for several years.

Seed preserved for about 20 years		
Species	**Scientific name**	**Year produced**
Rapeseed	*Brassica napus var. velox.*	1993
Alfalfa	*Medicago sativa*	before 1993
Birdsfoot trefoil	*Lotus corniculatus*	before 1993
Red clover	*Trifolium pratense*	before 1993
Orchardgrass	*Dactylis glomerata*	1993
White clover	*Trifolium repense*	before 1993
Perennial ryegrass	*Lolium perenne*	1990
Sorghum hybrid	*Sorghum bicolor x S. bicolor*	1993
Seeds preserved for several years		
Species	Scientific name	year produced
Garland chrysanthemum	*Chrysanthemum coronarium*	2006
Chard	*Beta vulgaris var. cicla*	2006
Nappa cabbage	*Brassica rapa*	2005
Spinach	*Spinacia oleracea*	2006
Young summer radish	*Raphanus raphanistrum*	2005

Table 2. Germination ratio of plants.

Plant condition	**Weight (g)**	**Total**	**Germinated**	**Ratio (%)**
Spinach	-	112	0	0
Red clover	-	200	0	0
Red clover	0.5247	260	0	0
Birdsfoot trefoil	0.249	150	0	0
White clover	0.1005	150	0	0
White clover	0.1115	173	0	0
Alfalfa	-	100	3	3
Sorghum	-	50	0	0
Garland chrysanthemum	0.750	100	50	50
Garland chrysanthemum	0.2839	110	50	50
Young summer radish	0.6617	54	1	2

to know viability of vegetable seeds preserved for several years after their harvest.

MATERIALS AND METHODS

Table 1 shows the Seed preserved for about 20 years and those preserved for several years. The herbage seeds were produced from the year 1991 to 1993, and the vegetable seeds from the year 2005 to 2006. Therefore, there was an interval of around 20 years. The date of this experiment was carried out for four weeks from March 14, 2013; three weeks from March 21, 2013; and one week from April 18, 2013. In addition, the number of seeds was from 50 to 260. After counting the seeds, they were placed in a soaked filter pater on Petri-dish, whose radius was approximately 10 cm. The petri-dish remained on the desk of the laboratory.

RESULTS AND DISCUSSION

From herbage seeds, only alfalfa (*Medicago sativa*) showed about 3 - 5% germination ratio for 20 years old seeds. The results are similar to that Park and Kim (2009). From the other herbage species, no germination was observed, but sometimes the decay of seeds were observed. However, some of the vegetable seeds preserved for several years showed high germination ratios. Table 2 shows germination ratio of plants. From the Table it is clear that older seeds germinates less than the new seeds preserved for several years. However, for example, young summer radish germinated only as much as 1 seed per 50 ones (Figures 1 to 5).

Figure 1. Germination of alfafa: 3-5 germinations among 100 seeds were observed.

Figure 2. Germination of sorghum hybrid. Seeds did not decay but there was no germination.

Figure 3. Germination of garland chrysanthemum. The seeds showed good germination.

Figure 4. Germination of nappa cabbage. The seeds showed a vigourous germination.

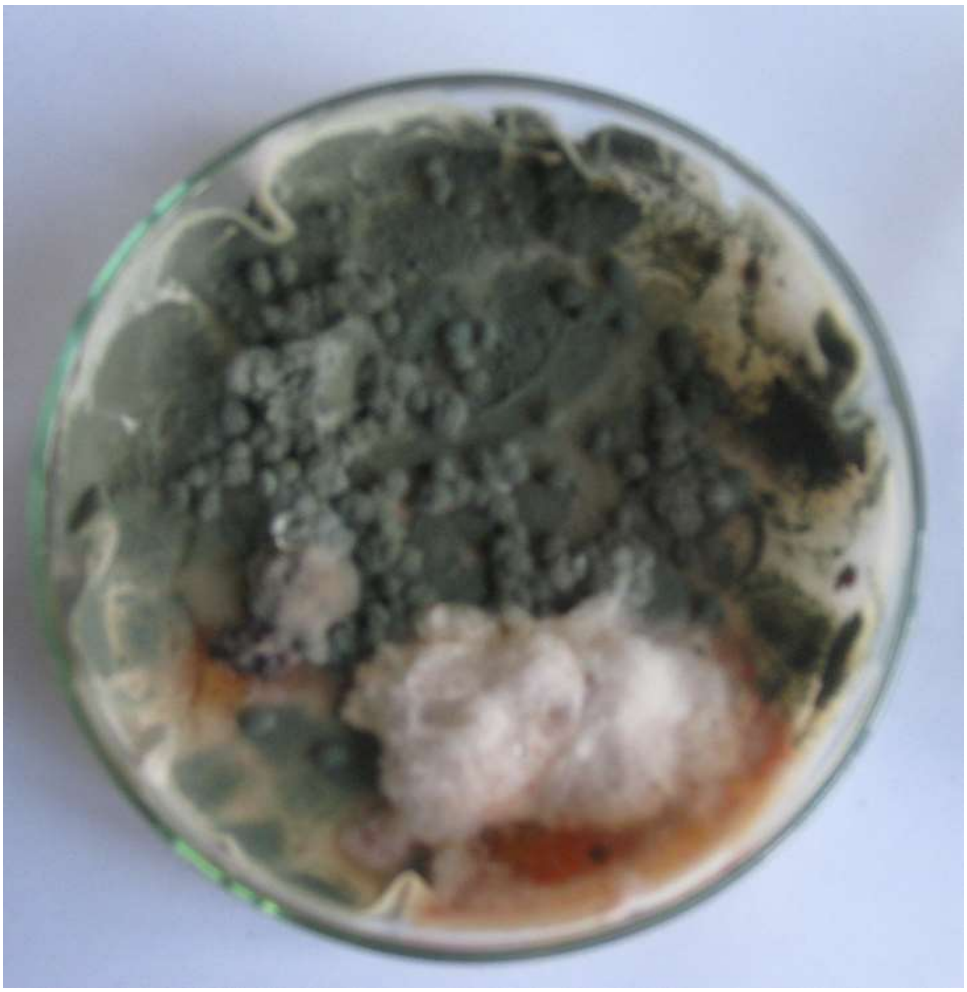

Figure 5. Germination of red clover. The red clover seeds showed whole decay during the germination. The reason of the decay seemed fungi.

Conclusion

As a conclusion, older seeds generally germinates slightly, while alfalfa seeds continue to live for long time. New seeds generally germinates more; whereas, young summer radish germinated a little.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Malt Barley (*Hordeum distichon* L.) varieties performance evaluation in North Shewa, Ethiopia

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Malt barley is the most important cereal crop grown in highland parts of Ethiopia. Even though Ethiopia has favorable environment and potential market opportunity, the share of malting barley production is quite low (about 15%) as compared to food barley. One reason for low production is the use of low yielding varieties. The present investigation was conducted in a randomized complete block design with three replications in Debre Berhan, Ethiopia, during 2015 and 2016 main cropping seasons to assess the performance of malt barley varieties for yield and yield related traits. Eight released and promising genotypes (Beka, EH1847, Bahati, Bekoji-01, Traveller, Holker, Sabini and Miskal-21) were evaluated. The mean square due to genotypes, year, and interaction effect were significant (P<0.05) for all traits studied except harvest index. Variety by year interaction effect also differed significantly for all characters except spike weight and harvest index. The highest yields were found from EH1847, Beka and Holker, (3.69, 3.53 and 3.72 ton/ha respectively) while the lowest yield (2.72 ton/ha) was recorded from Miskal-21. Variety EH1847 scored high yielding in both years hence, the use of either of EH1847 variety with full package for mass production in Debre Berhan and similar agroecology would increase malt barley production.

Key words: Evaluation, malt barley (*Hordeum distichon* L.), variety selection, yield, correlation.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the most staple food and subsistence crop in the country. It is grown in diverse environments with the altitude range of 1500 and 3500 masl, but predominantly grown from 2000 to 3000 masl (Berhane et al., 1996). It is the fifth most cultivated crop of the world (Eshghi and Akhundova, 2010). In Ethiopia, it is ranked fifth next to tef, wheat, maize and sorghum (CSA, 2017). In 2016/2017 cropping season the total area covered by barley in Amhara Region is about 323,600 hectares (CSA, 2017). Cultivated barley is normally divided into three subgroups; six-row (*H. vulgare*), two-row (*Hordeum distichon*) and the seldom cultivated intermediate (*Hordeum irregulare*).

Both two-row and six-row barleys are used for malting, but the best malt quality for beer is produced from two-row varieties. The international and national demand of

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Table 1. Description of malt barley varieties evaluated at Debre Berhan during 2015 and 2016 main cropping season in North Shewa zone, Ethiopia.

Varieties	Year of release	Released center	Grain yield (t/ha) at a time of release	Recommended agro-ecology zone
				Altitude (masl)
EH1847	2011	HARC	3.5-4.0	2300-2800
Traveler	2013	Heinken/HARC	20-40	2000-2600
Miscal-21	2006	HARC	2.5-4.6	1550-2850
Sabini	2011	KARC/HARC	2.5-3.0	2300-2800
Bahati	2011	KARC/HARC	2.5-3.0	2300-2800
Beka	1973	HARC	2.5-3.8	2300-2800
Bekoji-01	2010	KARC	3.5-4.0	2300-3000
Holker	1979	HARC	2.4-3.1	2300-3000

HARC: Holeta Agricultural Research Center, KARC: Kulumsa Agricultural Research Center Crop Variety Register (1995-2016).

malt barley is directly associated with the expansion of the brewery industries. In Ethiopia, malt barley is the major (90%) raw material for beer production (MoARD, 2010); hence the country faced a shortage of malt barley to meet the demand of the local breweries (Mohammed and Getachew, 2003). To fulfill the increasing malt barley demand, and to ensure higher cash return to the farmers, expansion of the malt barley production is very important. Even though Ethiopia has favorable environment and potential market opportunity, the share of malting barley production is quite low (about 15%) as compared to food barley. The local malt barley production covers about 35% malt demands; as a result the breweries are forced to import malt from abroad (Molla et al*.,* 2018). One reason for low production, particularly at Debre Berhan is the lack of improved malt barley varieties. To challenge the boosting demand and stunted supply of malt barley, it is important to make malt barley varieties evaluation. Hence, the present investigation is set with the objective of evaluating and selecting adaptable and high yielding malt barley variety (ies).

MATERIALS AND METHODS

The study was conducted at Debre Berhan during the main cropping season for two years (2015 and 2016). Debre Berhan is located in the North Shewa Zone of the Amhara Region, about 130 kilometers north east of Addis Ababa on the paved highway to Dessie, the town has a latitude and longitude of 9°41'N39°32'E and an altitude of 2840 masl. The annual average temperature and rainfall is 12.85°C and 927 mm respectively.

The experiment comprised eight nationally released malt barley varieties (Traveler, Sabini, Bekoji, Bahati, EH-1847, Beka, Holker, and Miskal-21) (Table 1). The varieties were laid out in Randomized Complete Block Design (RCBD) with three replications. The experimental plot contained ten rows of 2.5 m length with the spacing of 0.2 m between rows. The spacing between plot and blocks were 0.5 and 1 m respectively. Planting was carried out by hand drilling using seed rate of 100 kg/ha. Recommended fertilizer rate of 100 kg/ha Urea and 100 kg/ha NPS was used as per the package. During initial crop development weed management was carried out manually to avoid competition and weed interference.

Data collection and analysis

Data were collected on days to heading, days to maturity, biomass, spike length, spike weight, plant height, number of tiller per plant, spike weight, number of kernels per spike, thousand kernel weights, grain yield, and harvest index. The data were collected from eight middle rows. Replications and years were considered as random effects whereas varieties were considered as fixed effect. To reveal the total variability present within the tested varieties, the data were computed for all the characters measured as per Gomez and Gomez (1984). The data were subjected to analysis of variance (ANOVA) following statistical procedures appropriate for the experimental design using Statistical Analysis System (SAS) program package version 9.1. Whenever treatment effects were significant at 0.05 level of error, the means were delineated by using the least significant difference (LSD) procedures. Correlation analyses were determined through simple correlation coefficient between yield and other traits studied.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) was carried out to determine the main effect of varieties, years and their interaction on yield and yield components of malt barley varieties (Table 2). There were statistically significant ($P<0.05$) variation in varieties and years for all investigated traits except harvest index. Variety by year interaction also differed significantly for all characters except harvest index and spike weight. This result is in agreement with Daniel (2010), Yetsedaw et al. (2013) and Bankole et al*.* (2015).

The mean performances across the two years for all studied traits are presented in Table 3. Days to heading ranged from 78.17-88.17 days, and Sabini was early to heading whereas Beka was too late to head. It takes 125 days to mature for variety Sabini and for Beka 143.33 days. Maximum plant height (126.85 cm) was recorded for Beka, while minimum (81.18 cm) for Traveler. Bekoji-01 needs shortest (43.5) days to fill grain, whereas Traveler needs 56.33 days. Total biomass yield varied from 8.45 -11.92 tons per hectare with a mean of 11.42, and 11.92 tons per hectare was recorded for Bahati and

Table 2. Mean squares from combined analysis of variance for the effects of year, and variety on different parameters studied evaluated in 2015 and 2016 at Debre Berhan.

Traits	Year (Y) (Df = 1)	Rep.(R) (Df= 4)	Variety (V) (Df=7)	G*Y (Df = 7)	Error (Df=28	Mean	R^2	CV (%)
DH	75.00***	0.13^{ns}	63.38***	3.62***	0.34	82.50	0.98	0.71
GFP	221.02***	0.15^{ns}	111.35***	5.07***	0.41	50.48	0.99	1.26
DM	200.08***	0.77*	162.38***	1.85***	0.25	131.58	0.99	0.38
PH	921.38***	61.57^{ns}	1171.36***	325.59***	41.17	103.29	0.91	6.21
FTNPP	199.27***	1.40^{ns}	16.56***	15.82***	0.95	11.33	0.94	8.59
SL(cm)	1.66***	0.04^{ns}	3.15***	0.50***	0.03	7.57	0.97	2.35
NKPS	8.38*	0.70^{ns}	13.89***	8.06***	1.47	27.05	0.80	4.49
SW(g)	0.71***	0.01^{ns}	0.06*	0.25ns	0.02	1.37	0.68	10.87
TSW(g)	5143.95***	47.74^{ns}	102.53*	93.94*	34.64	83.55	0.87	7.04
TBY/Ha (ton)	5.93***	0.31^{ns}	9.75***	6.37***	0.37	10.10	0.93	6.00
GY/ha (ton)	5.65***	0.01^{ns}	0.70***	0.465***	0.026	3.30	0.95	4.93
HI	0.003	0.0002	0.005	0.004	0.0004	0.32	0.87	5.99

*, ** and *** = significant at P<0.05, P<0.01, and P<0.001, respectively. ns = nonsignificant difference, Days to Heading (DH), grain filling period (GFP), Days to maturity (DM), Plant Height (PH), Fertile tiller number per plant (FTNPP), Spike length (SL), Number of kernels per spike (NKPS), Spike weight (SW), Thousand seed weight (TSW), Total biomass yield (TBY), grain yield (GY), and Harvest Index (HI).

Holker varieties.

Number of kernels per spike was also significant (P<0.01) among varieties, maximum number of kernel per spike (27.76, 28.19 and 28.56) was recorded for EH1847, Beka and Bekoji-01 respectively. Similarly, reports have shown the variation of number of kernels per spike as a function of barley genotypes (Ryan et al., 2009; Biruk et al., 2016). In terms of fertile tiller number EH1847 and Beka give highest tiller number and they were statistically at par with the score of 13.52 and 12.6 respectively. The least fertile tiller producer variety was Miskal-21 (8.15). The longest spike length (8.8 cm) was recorded for Beka and shortest (6.58 cm) for Holker. Thousand kernel weight was significantly different among the varieties. This result agrees with those obtained by other authors (Rashid and Khad, 2008; Bagheri and Sadeghipour, 2009), who reported significant variation between malt barley genotypes in number of kernels per spike. The range observed for thousand seed weight was 79.5-89.97 g with overall mean of 83.5 g. Highest thousand seed weight (89.97, 88.68 and 86.15 g) was recorded for Behati, Holker and Bekoji-01 respectively, which is also in line with findings of Aliyi et al. (2016). The mean grain yield ranged from 2.72-3.72 tons per hectare. The highest grain yield (3.72, 3.69 and 3.53 t/ha) was recorded by Holker followed by EH1847 and Beka while the least was recorded by Miskal-21 (2.72 t/ha). The best yielding ability of EH1847 variety might be attributed to larger tiller number per plant, number of kernel per spike, and thousand seed weight. This is in line with Moral et al. (2002), who reported that the number of fertile tiller number per plant, number of kernel per spike and thousand seed weight are important features of cereals in determining the yield potential.

The result of variety by year interaction effect shows a highly significant difference indicating differential varieties performance in each year. The variation of rainfall amount and distribution of the years 2015 and 2016 might have contributed significantly to the differences observed between the years for the characters studied. Significant variety by year interaction has also been reported by different scholars (Afzal et al., 2011; Anley et al., 2013; Bankole et al., 2015). Most varieties perform better for plant height, fertile tiller number, thousand seed weight, total biomass and grain yield in 2016 compared to 2015 season (Table 4). Variety EH1847 is high yielding in both years, whereas Miskal-21 variety was the least.

Correlation between traits taken

Correlation analysis provides the information of interrelationship of important plant characters and hence leads to a directional model for direct and indirect improvement in grain yield (Khan et al., 2004). Correlation coefficient among yield and yield components is presented in Table 5. The correlation analysis indicates there was significant positive relation between grain yield and date of maturity, plant height, fertile tiller number, number of kernel per spike, thousand seed weight, and total biomass yield. A positive and highly significant (r = 0.604) correlation between grain yield and fertile tiller number was found, while a very weak but non-significant (r = 0.031) correlation between grain yield and spike length was also noticed. This suggests that grain yield will increase at longest maturity period, highest plant height, fertile tiller number, kernel number per spike, thousand seed weight, and total biomass yield. Total biomass yield correlated with date of heading, plant height, and number of kernel per spike (r = 0.423, r =

Table 3. Combined mean performance of malt barley varieties for yield and yield components evaluated in 2015 and 2016 at Debre Berhan.

Varieties	DH	GFP	DM	PH (cm)	FTNPP	SL (cm)	NKPS	SW (g)	TSW (g)	TBY/Ha (ton)	GY/ha (ton)	HI
Beka	88.17^{a}	51.83^{d}	140.33^{a}	126.85^{a}	12.60^{ab}	8.80^{a}	28.19^{a}	1.35^{b}	79.50^{c}	11.07^{bc}	3.53^{ab}	0.32^{de}
EH1847	79.17^{f}	52.83^{c}	129.33^{e}	98.12^{cd}	13.52^{a}	8.20^{b}	27.76^{a}	1.28^{b}	80.37^{c}	9.93^{d}	3.69^{a}	0.38^{a}
Bekoji-1	85.00^{b}	43.50^{h}	127.00^{g}	111.77^{b}	12.22^{bc}	7.09^{e}	28.56^{a}	1.35^{b}	86.15^{abc}	10.40^{cd}	3.15^{d}	0.31^{de}
Holker	83.83^{c}	50.00^{e}	133.17^{d}	108.90^{b}	11.83^{bcd}	6.58^{f}	26.08^{b}	1.40^{b}	88.68^{ab}	11.42^{ab}	3.72^{a}	0.33^{cd}
Travller	81.67^{d}	56.33^{a}	135.83^{b}	81.18^{e}	10.05^{e}	7.97^{c}	27.31^{ab}	1.38^{b}	79.93^{c}	8.98^{e}	3.17^{cd}	0.35^{bc}
Sabini	78.17^{g}	48.33^{f}	125.00^{h}	90.95^{d}	10.97^{de}	7.46^{d}	27.45^{ab}	1.32^{b}	81.47^{c}	8.45^{e}	3.07^{d}	0.36^{ab}
Miskal-21	83.17^{c}	46.33^{g}	127.67^{f}	108.65^{b}	8.15^{f}	7.49^{d}	23.75^{c}	1.29^{b}	82.37^{bc}	9.15^{e}	2.72^{e}	0.3^{ef}
Bahati	80.83^{e}	54.67^{b}	134.33^{c}	99.87^{c}	11.30^{cd}	6.94^{e}	27.28^{ab}	1.59^{a}	89.97^{a}	11.92^{a}	3.36^{bc}	0.28^{f}
Mean	82.50	50.48	131.58	103.29	11.33	7.57	27.05	1.37	83.55	10.10	3.10	0.32
R2	0.98	0.99	0.99	0.91	0.94	0.97	0.80	0.68	0.87	0.93	0.95	0.87
CV (%)	0.71	1.26	0.38	6.21	8.59	2.35	4.49	10.87	7.04	6.00	5.36	5.99

Table 4. Mean performance of some traits of malt barley varieties across two consecutive years.

Varieties	2015					2016				
	PH (cm)	FTNPP	TSW (g)	TBY/Ha (ton)	GY/ha (ton)	PH (cm)	FTNPP	TSW (g)	TBY/Ha (ton)	GY/ha (ton)
Beka	120.27^{a}	9.0^{abc}	69.77^{b}	10.53^{bc}	2.87^{bc}	133.43^{a}	16.2^{ab}	89.23	11.6^{ab}	4.19^{a}
Eh1847	88.0^{c}	9.83^{ab}	63.8^{b}	7.73^{d}	3.28^{a}	108.23^{bcd}	17.2^{a}	96.93	12.13^{a}	4.10^{a}
Bekoji-1	119.7^{a}	10.23^{ab}	79.9^{a}	9.73^{c}	3.08^{ab}	103.83^{cde}	14.20^{c}	92.40	11.07^{bc}	3.22^{cd}
Holker	107.9^{b}	10.43^{a}	80.9^{a}	11.33^{ab}	3.14^{ab}	109.9^{bc}	13.23^{c}	96.45	11.5^{abc}	3.7^{b}
Travller	66.1^{d}	8.67^{bc}	68.1^{b}	7.37^{d}	2.37^{e}	96.27^{de}	11.43^{d}	91.75	10.6^{cd}	3.97^{a}
Sabini	86.77^{c}	8.63^{bc}	69.17^{b}	7.2^{d}	2.7^{cd}	95.13^{e}	13.3^{c}	93.77	9.7^{de}	3.43^{c}
Miskal-21	100.13^{b}	9.43^{abc}	68.8^{b}	9.37^{c}	2.47^{de}	117.17^{b}	6.87^{e}	95.93	8.9^{e}	2.97^{e}
Bahati	102.37^{b}	8.1^{c}	85.17^{a}	12.27^{a}	3.15^{ab}	97.37^{cde}	14.83^{bc}	94.77	8.8^{e}	3.1^{de}
LSD	9.03	1.7	9.48	1.17	0.28	13.08	1.68	11.07	0.91	0.24

0.475 (P<0.01), and r = 0.293 (P<0.05) respectively).

Conclusion

The national demand of malt barley is increasing as a result of expansion of the brewery industries. However, malt barley production is unable to feed the malt barley market demand of the country. Lack of improved varieties leads greatly to low production in the study area. Development of superior crop varieties and/ or variety selection is the ultimate goal of the plant breeders to obtain higher grain yield to replace the existing low yielding malt barley varieties. Malt barley seed production can be enhanced through selecting varieties suitable for the area, which may result in varietal diversity for producers. The finding generated from the investigation discovered the presence of significant difference among

Table 5. Correlation coefficients of different traits of malt barley varieties combined over years.

	DH	DM	PH	FTN	SL (cm)	NKPS	SWt (g)	TSWt (g)	TBY (t/ha)	GY (t/ha)
DH	1									
DM	0.326*	1								
PH	0.704**	0.209ns	1							
FTN	0.338*	0.454**	0.227ns	1						
SL (cm)	0.078ns	0.078ns	0.059ns	0.089ns	1					
NKPS	0.177ns	0.186ns	0.145ns	0.599**	0.268ns	1				
SWt (g)	-0.207ns	-0.24ns	-0.142ns	-0.537**	-0.157ns	-0.13ns	1			
TSWt (g)	0.303*	0.573**	0.248ns	0.447**	-0.258ns	0.136ns	-0.328*	1		
TBY (ha/ton)	0.423**	0.088ns	0.475**	0.011ns	-0.071ns	0.293*	0.275ns	0.110ns	1	
GY (ha/ton)	0.182ns	0.335*	0.372**	0.604**	0.031ns	0.452**	-0.207ns	0.445**	0.331*	1
HI	0.017ns	0.262ns	-0.076ns	0.289*	0.106ns	-0.036ns	-0.399**	0.487**	-0.585**	0.131ns

*And ** = significant different at 0.05 and 0.01 level, respectively.

the varieties, and variety by year interaction for most parameters studied. On the bases of two years results of the study at Debre Berhan EH1847 performs best and maintains consistency in yielding potential in both years (3.69ton/ha). This variety also scored high value in number of fertile tiller number (13.52) and number of kernel per spike (27.76) which was highly correlated with grain yield. Therefore, authors recommend variety EH1847 for mass production; as a result small scale farmers can boost their income. However, further investigation is recommended on other improved varieties for stability over locations and years for yield and yield related traits since most of the customers were looking for high yielder, adaptable and stable malt barley variety.

Abbreviations

DH, Days to heading; **DM,** days to maturity; **PH,** plant height **SL,** Spike length; **GFP,** Grain filling period; **FTNPP,** Fertile tiller number per plant; **NKPS,** number of kernel per spike; **SW,** Spike weight; **TSW,** thousand seed weight; TBY/Ha, total biomass yield per hectare; **GY/Ha,** Grain yield per hectare; **HI,** Harvest index; **ANOVA,** Analysis of variance; **CV,** coefficient of variance;

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Calibration and validation of CERES-wheat in DSSAT model for yield simulation under future climate in Adet, North Western Ethiopia

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Crop models are highly useful for simulating crop and soil processes in response to variations in climate and crop management. However, well estimated crop genetic coefficients are required. So the purpose of this study is to calibrate and evaluate the performance of CERES-wheat model and to simulate the climate change impacts on phonological stages and grain yield of bread wheat (Tay and Senkegna varieties) in the study area. Observed climate data from National Meteorological Agency of Ethiopia from 1983 to 2015 and future climate from Climate Research Programme's Fifth Coupled Model Intercomparison Project (CMIP5) database across 20 Global Circulation Models for Representative Concentration Pathway (RCP4.5 and 8.5) emission scenarios in the time horizon of early-term (2010-2039), mid-century (2040-2069) and end-century (2070-2100) were used. Crop and soil data were obtained from Adet Agricultural Research Center. Decision Support System for Agro-technology Transfer (DSSAT) crop model was employed. There was strong agreement between the simulated and observed values with R^2 being 96, 79 and 79% for days to anthesis, grain yield and days to maturity, respectively for Tay wheat variety while 75% for days to anthesis, 92% for grain yield, and 75% for days to maturity of Senkegna bread wheat variety. On the other hand, during model validation, the goodness of fits (R^2) was 86% for anthesis day, 70% for grain yield and 96% for physiological maturity days of Tay wheat variety. Similarly for Senkegna bread wheat variety, R^2 was 89, 82 and 75% for anthesis day, grain yield, and physiological maturity days, respectively. The yield of both bread wheat varieties showed increase except in 2080s under RCP4.5 relative to the baseline. However, days to flowering and to maturity showed decreased in each time slice under both RCPs.

Key words: Calibration, validation, crop model, wheat, Ethiopia, East Africa.

INTRODUCTION

Evidence from the Intergovernmental Panel on Climate Change (IPCC, 2007a, b) is now convincing that climate change is real. Five Coupled Inter-Comparison Project (CMIP5) model predicted global mean surface temperatures for 2046-2065 and 2081-2100 likely to be in the ranges of 1-2 and 1-3.7°C, respectively relative to

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1986-2005 (IPCC, 2013). In sub-Saharan Africa, Ringler et al. (2010) reported high temperatures and mixed changes in rainfall by the year 2050, and would result in a decrease of wheat yields by -22% in 2050 year. Wheat is an important cereal crop grown in the highlands of Ethiopia (Schulthess et al., 1997). It has been among priority crops on research and development strategies in Amhara Region (ARARI, 2007). However, climate change would adversely affect wheat crop production and cause certain wheat growing areas to be no longer viable, and wheat species will be restricted to higher altitude of Ethiopia (Yumbya et al., 2011). The disrupting of the rainy season in Ethiopia alters national crop production by 90 to 95% (Kidane, 2010). Similarly, Osman and Sauerborn (2002) and Hagos et al. (2009) found the rainfall variability in Ethiopian leads to a 20% production deficit and increase in 25% poverty rate, which costs the economy over one-third of its growth potential. General Circulation Models (GCMs) are capable of simulating global climate and provide reliable representation to local level (Hassan, 2012). Agricultural Model Intercomparison and Improved Project (AgMIP) protocol is a worldwide cooperative effort linking climate, crop and socio-economic modeling to produce improved modeling capacity for integrated assessment of climate change impacts on the agriculture sector at local, regional and global scale (Rosenzwig et al., 2013). Habtamu et al. (2012) identified that the recent modeling studies are urgent to reach all vulnerable populations in Ethiopia under extreme climate condition. Belayneh (2011) reported that most climate models predicted that the temperature will increase over a period of time under future climate scenario in Ethiopia. Crop models are being used to evaluate the impact of climate change on crop production as a result of increased greenhouse gases (Rosenzweig et al., 1992; White et al., 2011). Crop models have also been used in inputs and resource management options for sustained agricultural production (Aggarwal et al., 1994; 2006). CERES-wheat is one of process oriented management level tool that has capacity to simulate the growth, development and yield of wheat under diverse environments in DSSAT model (Ritchie et al., 1998), which helps to enter data from field experiments, evaluate the models, estimate the generic coefficients of crop, conduct sensitivity analysis, analyze economic risk and uncertainty of alternative management options (Hoogenboom et al., 2010; Jones et al., 2003a).

Site specific calibration and validation of CERES-wheat model in specific soil and climate for a particular set of management inputs is needed for further application (Jones et al., 2010; Mavromatis et al., 2001). However, most wheat varieties in the study area are not introduced to the DSSAT model, and it is limited to assess the climate change impact on wheat yield production in Ethiopia in general and in Adet in particular. Most authors, such as Agnew and Chappel (1999), Woldeamlak (2009), and Dereje et al. (2012) assessed the effects of climate variability on yield in the study area. However, these studies correlate rainfall with yields, but not related during different stages of the crop growth to identify the critical effect at each stage of the crop growth, and limited to include more factors for yield production other than rainfall (like, soil properties and CO_2 concentration) (RIDA, 2011). Therefore, the objective of the study was to calibrate and evaluate the performance of CERES-Wheat model and simulating the climate change impact on wheat yield by using DSSAT model v 4.7.

MATERIALS AND METHODS

Description of study area

The study was conducted in Adet, North Western Ethiopia. It is found in Amhara Region and located at 11°16'N and 37°29'E with an altitude of 2216 m above mean sea level (Figure 1). The mean annual rainfall is 1250 mm, and the average annual maximum temperature is 25.5°C and minimum temperature is 9.2°C with the dominant soil types being Nitosol, Vertisols and Luvisols (AARC, 2006, 2012).

Data source

The climate data for the baseline period of 33 years from 1983-2015 were obtained from the National Meteorological Agency of Ethiopian (NMA), Bahir Dar branch for the study area. The data includes daily rainfall, minimum and maximum temperatures, wind speed, relative humidity, and daily solar radiation. Future climate data were generated from Climate Research Programme's Fifth Coupled Model Intercomparison Project (CMIP5) multi-model database systems across 20 GCMs under RCP4.5 and RCP8.5 emission scenarios for the time horizon of early-term (2010-2039), mid-century (2040-2069) and end-century (2070-2100) (AgMIP, 2013a, b). Crop management, phenological observations data were obtained from Adet Agricultural Research Center. Crop data includes maturity date, anthesis date and grain yield, while crop management data includes planting date, planting density and fertilizer application dates and rates. Soil data also obtained from Adet site soil profile (2006) in sample of four layers which are presented in Table 1. The full information was captured from field book and center annual report for 2000 to 2005 cropping season under rain-fed conditions.

Downscaling future climate data

Delta factors methods was employed to generate the daily data of rainfall, minimum and maximum temperatures, and solar radiation by perturbing the daily baseline data (1980-2015) using Agricultural Model Intercomparison and Improved Project (AgMIP) scenario generation scripts with R analytical tool (Diaz-Nieto and Wilby, 2005; Fowler et al., 2007; AgMIP, 2013c; IPCC, 2013). Climate models provide provision of future scenarios to assess the impact of climate change. The adjustment formula for modifying precipitation, maximum and minimum temperatures is stated as shown in Equations 1 and 2, respectively.

$$P_{adj,fur,d} = P_{obs,d}\ X \sum_{1=i}^{k} pi\left(\overline{P}_{GCM.fur,m/}\overline{P}_{GCM.ref,m}\right) \quad (1)$$

Table 1. Physical and chemical soil properties in Adet experimental site, North Western Ethiopia.

Parameter	Soil depth (cm)			
	0 - 30	30 - 90	90 - 140	140 - 200
Clay	66	69	68	64
Silt	24	15	18	24
Sand	12	16	14	12
Bulk density (g/cm3)	1.13	1.19	1.21	1.29
Organic carbon (%)	1.31	0.98	0.77	0.51
Total N (%)	0.11	0.09	0.06	0.04
pH	5.2	6.3	6.8	7.1
CEC (meq/100 g soil)	50.1	52	55.2	55.8

CEC: Cation exchange.

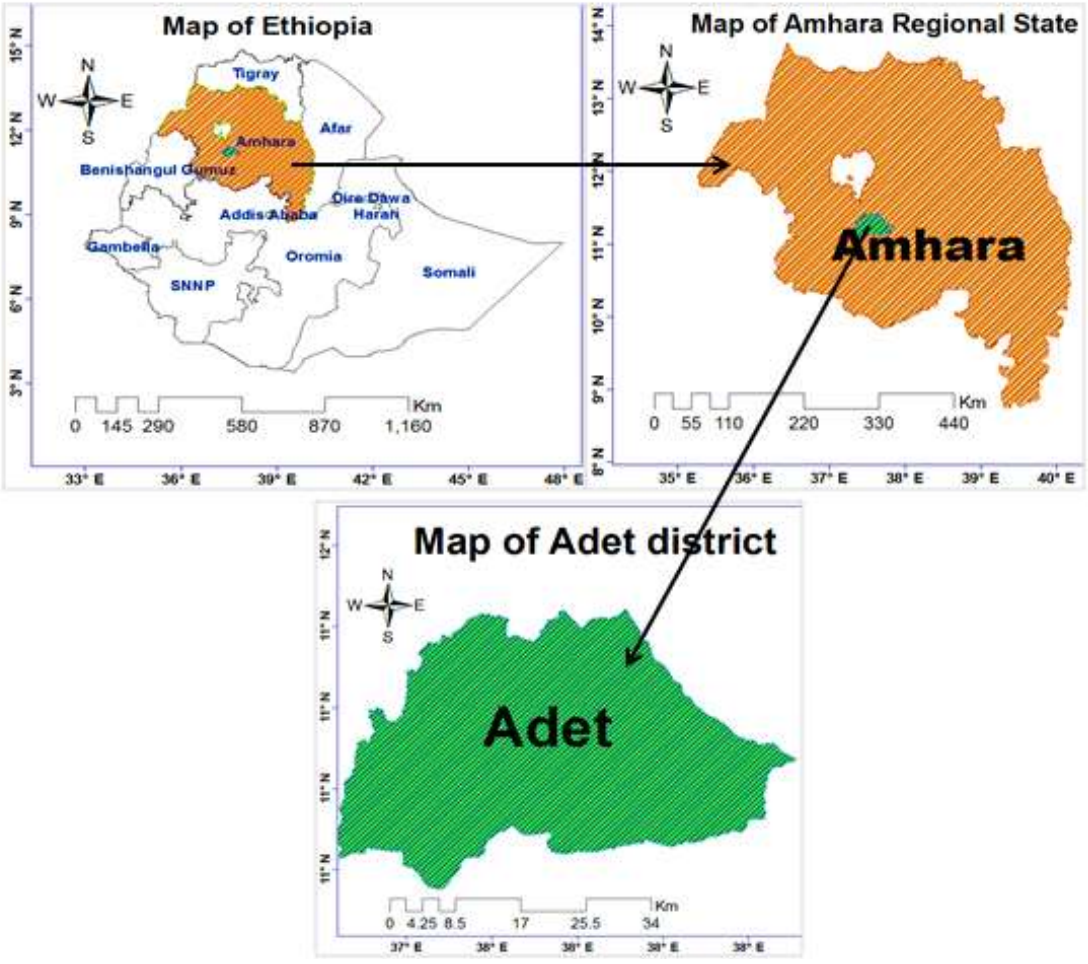

Figure 1. Location map of the study area

where $P_{adj,fur,d}$ was the adjusted daily rainfall for the future years, $P_{obs,d}$ was the observed daily rainfall for the base years, $\overline{P}_{GCM.fur,m}$ was the monthly mean rainfall of GCMs outputs for the future years, $\overline{P}_{GCM.ref,m}$ was the monthly mean rainfall of GCMs outputs for the base years, pi was the weight of each grid cell, and k was the number of grid cells.

Table 2. Genetic coefficients used to calibrate the CERES-Wheat model for Tay and Senkegna wheat variety in Adet, North Western Ethiopia.

Symbol	Definitions
P1V	Days, optimum vernalizing temperature required for vernalization
P1D	Photoperiod response (% reduction in rate/10 h drop in pp)
P5	Grain filling (excluding lag) phase duration (°C.d)
G1	Kernel number per unit canopy weight at anthesis (#/g)
G2	Standard kernel size under optimum conditions (mg)
G3	Standard non-stressed mature tiller wt (incl grain) (g dwt)
PHINT	Interval between successive leaf tip appearances (°C.d)

For temperature:

$$T_{adj,fur,d} = T_{obs,d}\ X \sum_{1=i}^{k} Pi\left(\overline{T}_{GCM.fur,m} - \overline{T}_{GCM.ref,m}\right) \quad (2)$$

where $T_{adj,fur,d}$ was the adjusted daily maximum or minimum temperature for the future years, $T_{obs,d}$ was the observed daily maximum or minimum temperature for the baseline years, $\overline{T}_{GCM.fur,m}$ was the monthly mean maximum or minimum temperature of GCMs data outputs for the future years, $\overline{T}_{GCM.ref,m}$ was the monthly mean temperature of GCMs outputs for the base years, pi was the weight of each grid cell, and k was the number of grid cells.

Crop model calibration

The calibration of CERES-wheat model utilized climate data for the baseline period of 33 years, crop data of 2000, 2001, and 2002 seasons for the most popular wheat varieties of Senkegna and Tay; and soil data on relevant parameters. GENCALC2 is software, which used to determine cultivar trait coefficients from data reported for an array of experiments. The model must use CULTIVAR and ECOTYPE files as specified for the DSSAT models, and must generate an EVALUATE.OUT file which conforms to the standards set up for DSSAT. Although this automatic calculation is a great deal of research to effective and efficient (Madsen et al., 2002), repeated iterations are needed until strong agreements occurred between the simulated and observed values. To initiate calibration, default genetic coefficients were created in WHCER047.CUL of DSSAT-CSM.

The derived genetic coefficients then used for model performance evaluation and finally for yield simulation. The seven genetic coefficients for model calibrations are shown in Table 2.

Crop model validation

In order to evaluate the calibrated model, well-defined criteria and input data are needed. Therefore, the performance of the CERES-wheat model was validated using an independent crop data from years that were not used for model calibration (2003, 2004 and 2005). The ultimate test of a simulation model is the accuracy which is usually involving comparisons between simulated and observed data (Willmott et al., 1985; Jones and Kiniry, 1986; Oreskes et al., 1994). A number of statistical methods for analyzing model performance are available. These are the root mean square error (RMSE) or percent of normalized root mean square error (RMSEn), index of agreement (d) (Willmott et al., 1985), and coefficient of determination (R^2) which is used for evaluating the goodness of fit between the observed and simulated values. Low values of RMSE and RMSEn, as well as d-values and R^2 close to unity is desired to define a good fit. The general formulas are summarized as:

$$RMSE = \sqrt{\sum_{i=1}^{n} \frac{(\mathrm{Pi} - \mathrm{Oi})^2}{N}} \quad (3)$$

$$RMSEn = \frac{100}{\bar{O}} \sqrt{\sum_{i=1}^{n} \frac{(\mathrm{Pi} - \mathrm{Oi})^2}{N}} \quad (4)$$

N was the number of observed values, Oi was observed, Pi was predicted values for the i^{th} data pair, and $\bar{O}$ was the overall mean of observed values. RMSEn (%) gives a measure of relative difference of simulated versus observed data. The index of agreement (d-static) provides a single index of model performance that encompasses bias and variability. The d-statistic was computed as:

$$d = 1 - \left[\frac{\sum_{i=1}^{n}(Pi - Oi)^2}{\sum_{i=1}^{n}(|Pi'| + |Oi'|)^2}\right] \quad (5)$$

where d was 0<d<1, and n number of observations, Pi was predicted value for the i^{th} measurement, and Oi observed value for the i^{th} measurement. $Pi' = Pi - \bar{O}$ and $Oi' = Oi - \bar{O}$. Finally, the simulations of yield and phenology have been examined by considering the CO_2 concentration (AgMIP, 2012). These carbon dioxide concentrations at baseline and each time period of 2030, 2050 and 2080 under RCP4.5 and RCP8.5 scenarios are shown in Tables 3 and 4. Similarly, the recommended fertilizer level, that is, 1.61 qt ha^{-1} Urea and 1.00 qt ha^{-1} DAP were applied. The calibration, validation as well as the simulation process was done with the nitrogen and water balance routine in model of DSSAT v 4.7.

RESULTS AND DISCUSION

Climate change projection

Future climate were downscaled at local level by using

Table 3. Mean change in projected climate between baseline (1983-2015) and future (2030-2080) under RCP4.5 in Adet, North Western Ethiopia.

Time slice	CO_2 concentration (ppm)	RF (%)	Max. Temp (°C)	Min. Temp (°C)
	RCP4.5	RCP4.5	RCP4.5	RCP4.5
2030	423	1	2.72	2.86
2050	449	2.16	2.11	2.16
2080	532	-0.35	2.69	2.86

Table 4. Mean change in projected climate between baseline (1983-2015) and future (2030-2080) under RCP8.5 in Adet, North Western Ethiopia.

Time slice	CO_2 concentration (ppm)	RF (%)	Max. Temp (°C)	Min. Temp (°C)
	RCP8.5	RCP8.5	RCP8.5	RCP8.5
2030	432	2.47	1.04	0.93
2050	571	2.92	2.76	2.51
2080	801	5.36	4.69	4.73

Table 5. Genetic coefficients for wheat in model and estimation results for both varieties in Adet, North Western Ethiopia.

Symbol	Minima in model	Maxima in model	Tay variety (ET-12D4/HAR-604)	Senkegna variety (HAR-3646)
P1V	0	60	9	10
P1D	0	200	25	31
P5	100	999	727	745
G1	10	50	42	41
G2	10	80	45	52
G3	0.5	8	2.8	2.3
PHINT	30	150	139	135

Equations 1 and 2 and then further analyzed by using R analytical tool. The relative changes of future rainfall and temperatures comparing to the baseline period (1983-2015), and the corresponding CO_2 concentration at each time period are shown in Tables 3 and 4 for RCP4.5 and 8.5, respectively. The temperatures for 2030-2080 is expected to rise in the ranges of 2.11-2.72 and 2.16-2.86°C in maximum and in minimum temperature, respectively, while the rainfall increase by 1 to 2.16% in 2030 to 2050, but decrease by 0.35% in 2080s under RCP4.5 (Table 3). Similarly, for RCP8.5, maximum and minimum temperatures showed increase in the range of 1 to 4.6°C and 0.93 to 4.73°C, respectively, and the rainfall showed increase by 2 to 5% in 2030 to 2080 time periods (Table 4). The positive change in temperature indicates it will be warmer than today. IPCC (2014a) indicated that large scale increase in average temperature in the mid and late 21st century. If the increasing temperature is not offset by adequate moisture, the intensity and duration of drought might increase, and results in failure of bread wheat production. For instance, wheat yield has been predicted to decrease approximately 3 to 4% for each 1°C rise in temperature above 15°C during the grain filling period (Wardlaw and Wrigley, 1994). You et al. (2009) carried out research in China and found out that an increase in temperature of 1°C during the growing period may lead to yield reduction by 3 to 10%. Flato et al. (2013) noted, future climate projection is quite uncertain and the output depends on the number and type of climate models used.

Model calibration

The parameters for the specific crop are shown in Table 5. During the model calibration, the crop developments or phenologies were more sensitive to the genetic coefficients of P1V, P1D, and P5; crop growth or yield attributes were more sensitive to G1, G2, and G3 coefficients.

The calibration results are satisfactory as depicted in Table 6. Strong agreement is shown between the

Table 6. Statistical indicators during model calibration for both bread wheat varieties in Adet, North Western Ethiopia.

Statistical parameter	Senkegna variety			Tay variety		
	Anthesis day	Yield (qt ha^{-1})	Maturity day	Anthesis day	Yield (qt ha^{-1})	Maturity day
Observed	66	36.7	120	69	36.5	119
Simulated	70	29.1	120	73	28.7	122
R^2 (%)	75	92	75	96	79	79
d-stat (%)	51	62	76	57	60	52
RMSE	4	7.3	1.3	4	8.2	3
RMSEn (%)	6.8	19.8	1	6.1	22.5	2.6

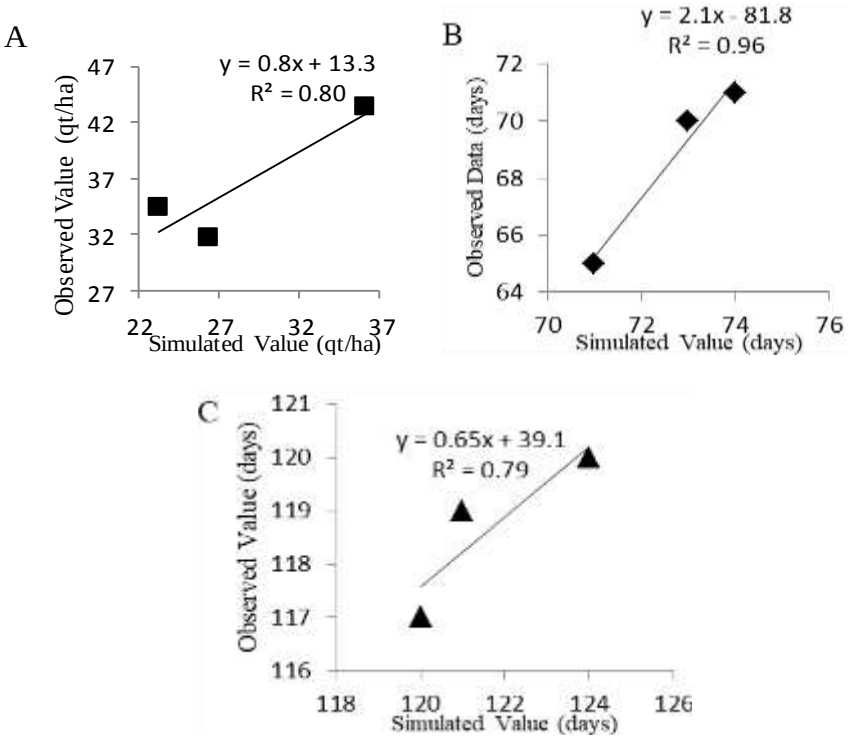

Figure 2. Relationship between simulated and observed value of grain yield (A), days to anthesis (B), and days to maturity (C) in calibration for Tay bread wheat variety.

simulated and observed values with R^2 of 96, 79 and 79% for days to anthesis, days to maturity, and maturity yield, respectively for Tay wheat variety, 75% for days to anthesis, 75% for days to maturity, and 92% for maturity yield for Senkegna wheat variety. The overall performance for Tay wheat, the simulation of days to anthesis, was found to be good as compared to the yield and maturity days, and there was good agreement.

The regression coefficients, that is, 0.8 for grain yield, 2.1 for days to anthesis and 0.65 for days to maturity indicated a good association between observed and simulated values for Tay wheat variety (Figure 2). Similarly, for Senkegna wheat, the regression coefficients of 0.67, 1.5 and 0.37 for grain yield, days to anthesis and days to maturity, respectively showed strong association between the observed and simulated values (Figure 3). The 1:1 line graph showed observed yield in Y-axis and simulated yield in X-axis. The amount of R^2 resulted from analysis of linear regression of the functions closer to 1, which indicates the model description for yield simulation is better (Reza et al., 2005).

Model validation

As depicted in Table 7, the validation results are also satisfactory. The model had strong performance for Tay wheat during model evaluation with the goodness of fits

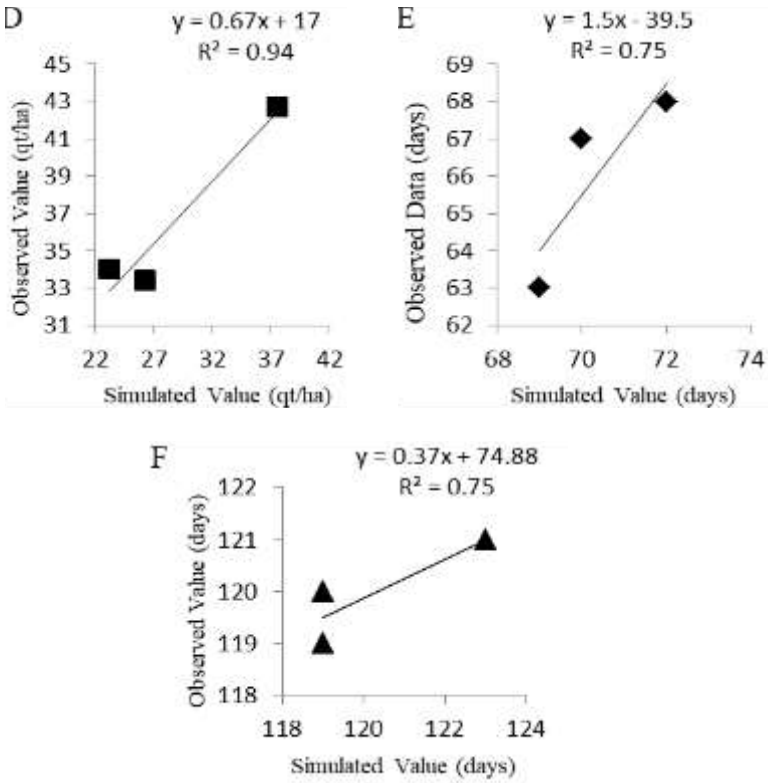

Figure 3. Relationship between simulated and observed value of grain yield (D), days to anthesis (E), and days to maturity (F) in calibration for Senkegna bread wheat variety.

(R^2) as 86, 70 and 96% for anthesis day, grain yield and maturity days, respectively, while 89, 82 and 75% for anthesis day, grain yield, and maturity days, respectively for Senkegna wheat. The corresponding observed index agreement statistics (in Equation 5) also supports the same results for days to anthesis and days to maturity (d>51%), but lower for grain yield (d=42%) for Tay wheat, and good agreement for all parameters ranged between 52 and 76% for Senkegna wheat. Table 7 also shows the RMSEn (in Equation 4) indicated the excellent agreement between simulated and observed value by 4.1% for anthesis days, fair agreement by 20.2% for grain yield, and good agreement by 12.5% for physiological maturity for Tay wheat (Valizadeh et al., 2013). Similarly, the RMSEn for Senkegna wheat showed excellent agreement by 6.2 1.4% for days to anthesis and grain yield, respectively while good agreement for days to maturity by 17.8% (Valizadeh et al., 2013). The study showed high RMSEn for grain yield as compared to other parameters, and revealed low in model performance (Table 7). Rezzoug et al. (2008) used DSSAT model to calibrate and validate 9 wheat cultivars in Algeria, and predicted final yield with RMSE of 7.6 qt ha^{-1} and R^2 of 0.71.

Therefore, well calibrated and validated CERES-wheat model can be ready for applications such as prediction of crop growth, phenology, water management, potential and actual yields, and generating agronomical adaption options.

Simulation of yield and phenology under future climate

The yields of both wheat varieties in response to future climate are expected to increase from 2030 to 2050, except in 2080s under RCP4.5 for Tay wheat (Table 8). However, the days to anthesis and days to maturity of both wheat varieties become decline. Wheat yield and growth are influenced by CO_2. For instance, doubling of ambient CO_2 has been reported to cause an approximate 40% decrease in stomatal space, which may reduce transpiration by 23 to 46% (Cure and Acock, 1986; Morison, 1987), and might cause a 10 to 50% increase in growth and yield of C_3 crops. Similarly, doubling of CO_2 concentration enhances photosynthetic rate of leaves by 25 to 50% and adds up to the increase in photosynthetic yielding and plant productivity up to 30 to 60% (Mulholland et al., 1997). On the contrary, for each temperature increase in mean air temperature during grain filling in wheat, the duration of grain filling was shortened by 3.1 days and final kernel weight was

Table 7. Statistical indicators of model performance of wheat in Adet, North Western Ethiopia.

Statistical parameter	Senkegna variety			Tay variety		
	Anthesis days	Yield (qt ha^{-1})	Maturity day	Anthesis days	Yield (qt ha^{-1})	Maturity days
Observed	67	32.4	119	65	34.1	116
Simulated	70	27.1	120	72	28	121
R^2 (%)	0.89	0.82	0.75	0.86	0.7	0.96
d-stat (%)	0.47	0.51	0.84	0.51	0.42	0.52
RMSE	4.2	5.7	1.5	8	6.9	5
RMSEn (%)	6.2	17.8	1.4	12.5	20.2	4.1

Table 8. Days to anthesis (DTA), maturity (DTM) and grain yield of Tay and Senkegna bread wheat varieties under baseline and future climate in Adet, North Western Ethiopia.

Scenario	Tay variety			Senkegna variety		
	DTA	DTM	Yield (qt ha^{-1})	DTA	DTM	Yield (qt ha^{-1})
Baseline	70	119	38.20	71	121	38.79
RCP4.5_2030	66	112	39.05	67	114	39.43
RCP4.5_2050	62	106	38.25	63	108	39.23
RCP4.5_2080	60	102	38.48	61	104	39.78
RCP8.5_2030	66	112	38.81	67	114	39.18
RCP8.5_2050	61	103	38.47	62	105	39.68
RCP8.5_2080	55	94	36.10	56	95	39.11

DAT: Days to anthesies, DAM: days to maturity.

reduced by 2.8 mg (Wiegand and Cuellar, 1981). These results suggest that an increase in temperature may offset the benefits of increasing CO_2 on crop yield. Wolf et al. (2005) reported that temperature increase would result in yield reduction whereas increase in the level of precipitation and CO_2 fertilization would have positive impact on the production of wheat in Europe. As shown in Table 3, the CO_2 concentration is increasing. Thus, the elevated CO_2 increases carbohydrate pools of leaves and stems, and finally to grain yield (Attri and Rathore, 2003). Evidence wheat production will increase by 25% in Mexico region (Lobell et al., 2005), and increase by 3.1 and 4% at low high altitude, respectively up to 2030s (Xiao et al., 2005).

Grain yield variability under the baseline and future climate

For Tay wheat variety, 25% of the yield results ranged between 32 and 34 qt ha^{-1} and 75% of the yield results ranged between 41 and 44 qt ha^{-1} in time horizon of 2030 to 2080 under both RCPs (Table 9). For Senkegna wheat variety, 25% of the yield results ranged between 34 and 39 qt ha^{-1}, and 75% of the yield results ranged between 42 and 45 qt ha^{-1} in time horizon of 2030 to 2080 under both RCPs (Table 10). The variability of yield (coefficients of variations ranged between 14 and 17%) under baseline and future climate showed less varied thought, the time period for both bread wheat varieties (Hare, 1983).

Conclusion

The study involved through calibrating and validating of the model and simulating of wheat yield and phenology under baseline and future climate. The overall calibration and validation of CERES-wheat model has good agreement between the observed and simulated value of days to anthesis, days to maturity and yield and for both wheat varieties and revealed suitable further applications, such as in prediction of crop growth, phenology, potential and actual yields. Although future climate changes have positive and negative impact on Tay wheat, the grain yield will increase relative to the baseline for both wheat varieties. On the other hand, the simulation days from planting to flowering and to maturity of the two wheat varieties were reduced.

LIMITATIONS

The general aim of the CERES- wheat model calibration and validation study was to bring the model performance in approach to 100% agreement and applicable for further agricultural decision making for the particular crop varieties in the specific environment including this study.

Table 9. Variability grain yield (qt ha^{-1}) for Tay wheat at different time slice under RCP4.5 and RCP8.5 scenarios in Adet, North Western Ethiopia.

Statistical parameter	Baseline	RCP4.5			RCP8.5		
		2030	2050	2080	2030	2050	2080
Minimum	30.57	31.02	31.21	26.11	30.21	27.80	18.03
1st quadrant	32.89	32.97	33.84	33.68	32.66	33.26	33.48
Median	36.82	37.86	36.61	37.48	37.63	37.97	35.39
3rd quadrant	42.81	43.60	41.67	43.75	43.59	43.12	40.08
Maximum	49.13	49.19	50.14	49.48	49.77	49.05	45.42
Mean	38.20	39.05	38.25	38.48	38.81	38.47	36.10
SD	59.7	6.11	5.70	5.95	6.49	5.98	5.37
CV (%)	15.6	15.6	14.9	15.5	16.7	15.5	14.9

Table 10. Variability of grain yield (qt ha^{-1}) for Senkegna whea at different time slice under RCP4.5 and RCP8.5 scenarios in Adet, North Western Ethiopia.

Statistical parameter	Baseline	RCP4.5			RCP8.5		
		2030	2050	2080	2030	2050	2080
Minimum	29.37	31.03	31.10	29.08	30.12	28.70	22.89
1st quadrant	33.26	34.81	33.49	39.43	32.96	33.47	34.75
Median	36.73	39.30	38.21	38.24	37.97	38.41	38.80
3rd quadrant	43.85	44.57	42.08	42.20	44.68	44.85	43.34
Maximum	51.15	49.94	51.41	51.28	50.26	50.36	49.57
Mean	38.79	39.43	39.22	39.78	39.18	39.68	39.11
SD	6.47	6.14	6.6	6.55	6.72	6.63	5.86
CV (%)	16.7	15.6	16.8	16.5	17.2	16.7	15.0

However, this might be achieved as a result of high quality data, which was obtained from well managed fields and well calibrated model used.

RECOMMENDATION

Crop models need reliable data inputs and able to generate more relevant climate information for decision making and adaptations measurements in agriculture. Therefore, building of data archive capacity, such as network of weather stations, soil database, and crop phenological observation should be established in order to promote soil-crop-climate research.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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