INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 12, No. 3, p. 131-146, 2018

REVIEW PAPER OPEN ACCESS

Salinity effects on wheat (*Triticum aestivum L.*) characteristics - a review article

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Key words: Salinity stress, wheat physiology, anatomical characteristics, molecular and genetical characteristics, yield and yield components.

http://dx.doi.org/10.12692/ijb/12.3.131-146 Article published on March 18, 2018

Abstract

Plants are subjected to numerous abiotic stresses which adversely influence on their growth, metabolism and productivity. Among them, salinity stress is one of the most detrimental factor for plants growth and yield.In present review, we describe the impacts of salinity stress on numerous characteristics of wheat crop including morphological (plant height, root length, number of leaves, leaf area, root hairs count) and physiological (water relations, stomatal conductance, photosynthesis and chlorophyll content) are some of them. Other characteristics like biochemical, molecular and anatomical aspects have also been addressed. It can actually be not easy to recognize which characteristics are the mainly imperative ones for salts-tolerance in a wheat system. For easiness to this complexity, authors suggest the graphs generation to assist in showing relationships among the traits especially biochemical and molecular characterizations that are of prime consideration to modify existing or/and develop new varieties.

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Introduction

Soil salinity is one of the most common abiotic stresses which tremendously decrease the productivity of agricultural crops in arid and semi-arid regions of the world (Hussain *et al.*, 2011). It occupies a prominent place among the soil problems that threatens the sustainability of agricultural crops over a vast area (Flowers, 2004). Apparently, 60% loss of the probable crop production is caused by salt stress in the environment (Xie *et al.*, 2016). Study depicts that more than 20% of the total cultivated land is affected by salinity stress (Oproi and Madosa, 2014).High level of soil salinity badly affects the quality and quantity of crop production (Akbari *et al.*, 2016) by preventing seeds germination, seedlings growth and developmental phases owing to the collective effects of high osmotic potential and specific ion toxicity (Muhammad and Hussain, 2012; Akbari *et al.*, 2016).

Soil salinity or salinization is generally referred as the addition of soluble salts into soils (Bockheim and Gennadiyev, 2000). It is mainly privileged in arid as well as semi-arid environments with evapo-transpiration degrees being higher compared with precipitations volume through the year. Salts in soils may increase naturally in the subsoil (primary soil salinity) or perhaps it be introduced due to anthropogenic (secondary soil salinity).It could be arise by soil modifications, fertilizers and irrigation by means of saline water (Carillo *et al.*, 2011).

Triticum aestivum (wheat) is the most important cereal reap of Pakistan and is cultivated all-over the country. Wheat is cultivated to rally the demands of food for population consumptions in Pakistan and others countries of world. However, per hectare productivity of wheat is so far lower as its production potential that is due to different factors and soil salinity is the most important one(Khan *et al.*, 2006). In present review article salinity effects on wheat different characteristics including germination, seedlings growth, morpho-physiological, anatomical, biochemical, molecular and genetical have been comprehensively discussed. As per authors' knowledge, there is not still a single, merged, constructive review available about salinity impacts on wheat's characteristics.

Salinity effects on wheat

Salinity stress shows elevated extent of correspondence in respect of seed germination, morpho-physiological, anatomical, molecular, biochemical and genetical impacts. This is probably attributable to the prove that sub-fatal saline condition is the first and foremost osmotic influence apparently analogous to that one brought in plants by water scarcity and heat stress.

Germination and seedlings growth

Salinity stress is one of the major problem affecting plant's seedlings, development and finally their productivity. Natural flora exhibit relatively differences in their salt stress tolerance capacity (Naz *et al.*, 2010). According to the findings by Hampson *et al.* (1990), Almansouri *et al.* (2001) and Saboora *et al.* (2006) , the saline conditions increased the time (Days) for seeds germination in difference wheat cultivars as compared to the non-saline conditions (Control). Elevated level of salts progressively increased the time to sprouting and decreased the sprouting percentage and affected the seedlings growth (Begum *et al.*, 1992; Sairam *et al.* 2002; Akbari *et al.,* 2007; Ghiyasi *et al.*, 2008). Reports by Arfan *et al.* (2007) andkhan *et al.* (2009) showed that salinity stress suppressed and delayed the germination of seeds in different wheat cultivars. Similar results were also reported by Mozafar *et al.* (1986), Afzal *et al.* (2005) and Iqbal *et al.* (2007) in wheat cultivars.

Morphological characteristics

Wheat crop acquires special consideration because of its morphological characteristics at all stages of growth in saline conditions which include leaf (shape, size, area, expansion, senescence, pubescence, cuticle tolerance and waxiness), root (length, root hairs, root area, fresh and dry weight, density) and vegetative (Plant height, diameter and fresh and dry biomass) (Table 1) (Kingsbury *et al.*, 1984; Rawson *et al.*, 1988;

Munns *et al*., 195). Thus, understanding wheat's responses to salinity stress at each phase of growth is essential to improvement in developing salt tolerant varieties with genetic modifications, and breeding techniques. Ahmad *et al*. (2013) found that early wheat maturity, reduced plant length, and decreased leaf area is linked to salinity stress. Kingsbury *et el.* (1984) and Bacilio *et al*. (2004) reported that the plant length, leaf expansion and flag leaf area in wheat was significantly lesser under salinity stress. According to the study of Zheng *et al*. (2008),salinity stress decreased the plant leaf area which as a result rate of photosynthesis was reduced that resulted in low biomass production. Furthermore, numbers of leaves/ plant and leaf size along with leaf longevity were shrunk as a result of salinity stress (El-Hendawy *et al*., 2009).

Table 1. Research scenario of morphological characteristics in wheat under salinity stress.

Morphological characteristics	Reference(s)
Plant size and stem diameter	(Kingsbury *et al*., 1984; Rawson *et al*., 1988; Munns *et al*., 1995; Ahmad *et al*., 2013)
Leaf area	(Kingsbury *et al*., 1984; Rawson *et al*., 1988; Munns *et al*., 1995; Bacilio *et al*., 2004)
Root extension	(Sabooraet *al*.,2006;Ahmad *et al*., 2013)
Roots length ,dry weight and density	(Sabooraet *al*., 2006;Ahmad*et al*., 2013)
Early maturity	(Kingsbury *et al*., 1984; Rawson *et al*., 1988; Munns *et al*., 1995)
Yield	(Saboora *et al*., 2006; Munns *et al*., 2006;Ali *et al*., 2008, Ahmad *et al*., 2013)
Leaf extension	(Bacilio *et al*.,2004;Ahmad *et al*., 2013
Leaf size	(Bacilio *et al*., 2004; Ahmad *et al*., 2013
Leaf number	[El-Hendawy *et al*., 2009; Ahmad *et al*., 2013)
Leaf longevity	(Bacilio *et al*., ;2004; El-Hendawy *et al*., 2009; Ahmad *et al*., 2013)
Root-to-shoot ratio	(Ahmad *et al*., 2013)

Table 2. Research scenario of physiological characteristics in wheat under salinity stress.

Physiological characteristics	Reference(s)
Stomata closure	(Kingsbury *et al*.,1984; Rivelli *et al*., 2002)
Cell wall integrity	(Moud *et al*., 2008; Mehta *et al*., 2010)
Metabolites production	(Fischer *et al*., 1998; Munns *et al*., 2006)
Oxidative stress	(Fischer *et al*., 1998; Munns *et al*., 2006)
Photosynthesis rate	(; Munns and Termaat, 1986;Goudarzi *et al*., 2008; Khan *et al*., 2009)
Turgor pressure	(Goudarzi *et al*., 2008; Khan *et al*., 2009)
CO_2 concentration	(Goudarzi *et al*., 2008; Khan *et al*., 2009)
Growth rate	(Munns *et al*., 2006; Khan *et al*., 2009)
Osmotic adjustment	(Khan *et al*., 2009;)
Stomata Co2 conductance	(Munns *et al*., 2006; Khan *et al*., 2009)
Relative water content	(Khan *et al*., 2009)
Membrane integrity	(Mehta *et al*., 2010)
Transpiration rate	(Khan *et al*., 2009)
Water use efficiency	(Munns *et al*., 2006)
Transpiration efficiency	(Goudarzi *et al*., 2008; Khan *et al*., 2009)
Total biomass	(Goudarzi *et al*.,2008; Khan *et al*., 2009)

Root is actually the first important organ since it has the potential to shift in order to salt avoidance (Naz *et al*., 2010) .Under stress conditions, production of ramified root system is vital to above ground biomass.

The significance of well developed root systems in absorbing water has been long known. (Zhu and Kang, 2003; Egamberdieva, 2009). A developed root system can contribute advantages to maintain plant growth for the period of early growth phases and extracts water and micro-nutrients through the soil. Sabooraet *al.* (2006) observed in his study that root development was more inclined to salinity stress in wheat and reduced root length as well area. The diverse escalation response of roots to saline environment is the adjustment to salt conditions for their survival (Arfan *et al.*, 2007; Akbarimoghaddam *et al.*, 2011). In conclusion, increased level of salt concentrations adversely affects root length and its viability to absorb water.

Table 3. Research scenario of biochemical characteristics in wheat under salinity stress.

Biochemical characteristics	Reference(s)
Proline, glycine betaine and polyols	(khan *et al.,* 2009; Reis et al., 2012)
Superoxide Dismutase (SOD)	(Sairam *et al.*, 20020
Catalase (CAT)	(Sairam *et al.*, 2002)
Polyamines (PAs)	(Foyer and Fletcher, 2001, Johnson *et al.*, 2003)
Reactive oxygen species (ROS)	(Rivelli *et al.*, 2002; Fazeli *et al.*, 2007)
Abscisic acid (ABA)	(Close *et al.,* 1996;Noaman et al.,2002)
Ions homeostasis	(Moustafa *et al.,* 1966; Gorham *et al.,* 1990; Rivelli *et al.*, 2002)
Chlorophyll content	(Khatkar *et al.*, 2000; James *et al.*, 2002)

Physiological characteristics

Salinity interrupts wheat's physiology at both cellular and whole plant levels through osmotic and ionic stress. Physiological processes which are harshly affected by salt stress comprise of alteration in plant development, mineral distribution, and membrane variability resulting from calcium dislocation by sodium and membrane permeability (Moud *et al.*, 2008; Mehta *et al.*, 2010). High salt concentration in soils decreases wheat leaf water potential, turgor pressure loss, stomata closure, decline in CO2 conductivity through stomata , alteration in cell wall integrity, increase of oxidative stress and enhances development of toxic metabolites which ultimately lead to plants' death (Kingsbury *et al.*,1984;Munns and Termaat, 1986; Rivelli *et al.*, 2002; Ali *et al.*, 2008; Taiz *et al.*,2015). It detrimentally affects the plants photosynthetic activity that results in low yield production. Reduced rate of photosynthesis in plants is one of the main causes behind decreased productivity (Goudarzi *et al.*, 2008; Khan *et al.*, 2009).

Table 4. Research scenario of molecular and genetical characteristics scenario in wheat under salinity stress.

Molecular and genetical characteristics	Reference(s)
Proline	(Singh *et al.*, Elshintinawy and Elshourbagy,2001;Wanget *al.*,2007)
SOD gene expression	(Wu *et al.*,1999)
CAT gene expression	(Sairam *et al.*,2002)
Dehydrins	(Close *et al.,* 1996)
Vacuolar acid invertase	(Vargas *et al.*, 2007)
Late embryo abundant (LEA)	Noaman *et al.*, 2002)
Osmotin	(Singh *et al.*,1987)
Glutathione S-transferase	(Anderson *et al.*,2004)
26 kDa protein	(Elshintinawy and Elshourbagy, 2001)
Early salt induced (ESI) gene	(Noaman *et al.*, 2002)
Abscisic acid (ABA)	(Noaman *et al.*, 2002)
Atls1 and α-tubulin genes	(Temel*et al.*,2015)

Through salinity stress, the stomatal pattern of gases conductance reduces and lead to inadequate availability of CO2 to the wheat plants (Table 2). Low CO2 concentration changes the rubisco; a photosynthesis key enzyme functioning as oxygenase instead of carboxylase and leads to more reactive oxygen species (ROS) production (Fischer *et al.,* 1998; Munns *et al.*, 2006). Reactive oxygen species exert a lot of detrimental effects impairing many functions such as retarded antioxidant defense system (James *et al.*, 2002; Bacilio *et al.*, 2004). Chlorophyll as well as caratinoides pigments content are reduced due to salt stress (Saboora *et al.*, 2006; Ali *et al.*, 2008). Chlorophyll and caratinoides amount correlate with the crop yield under salinity stress (Kong *et al.*, 2001).

Biochemical characteristics

Wheat crop exposures to the adverse environmental condition such as salinity stress increases the formation of reactive oxygen species (ROS) (Sairam *et al.*, 2002; Wimmer *et al.*, 2003; Wahid *et al.*, 2007). Generally ROS are produced in thalakaloid, mitochondria and peroxisomes during photosynthetic process, electron transport chain and glyoxylate cycle respectively (Moller, 2001; Reddy *et al.*, 2004, Fazeli *et al.*, 2007).

Table 5. Research scenario of yield characteristics in wheat under salinity stress.

Yield characteristics	Reference (s)
Grains protein content	(Mozafar *et al.*, 1986; Hina *et al.*, 2011)
spike length	(Irfan *et al.*, 2005; Haque, 2006; Hina *et al.*, 2011)
Number of spikes	(Irfan *et al.*, 2005; Haque, 2006; Hina *et al.*, 2011)
kernel weight and diameter	(Acevedo *et al.*, 2002; Irfan *et al.*, 2005)
Biological yield	(Forster *et al.*, 1988; Acevedo *et al.*, 2002; Irfan *et al.*, 2005)
Grain yield	(Sonia *et al.*, 2013; Hassan, 2015)
1000 grains weight	(Sonia *et al.*, 2013; Hassan, 2015)
seed number	(Irfan *et al.*, 2005; Hassan, 2015)
Number of tillers	(Hassan, 2015 ;Haque, 2006; Hina *et al.*, 2011)

The plants have enzymatic mechanisms intended for scavenging of salinity-induced ROS. The enzymatic systems are established to curtail the meditation of ROS. So far, the enzymes which are overproduced comprise of superoxide dismutase (SOD), glutathione reductase (GR), glutathione synthetase and ascorbate peroxidase (APX). Increase SOD, GR and APX activities under salinity stress and relatively higher activity in salinity-tolerant wheat varieties have also been accounted by Sairam *et al.* (2002). Continuously overproductions of ROS retard the antioxidant defense system of plants. ROS exert damaging effects on the macromolecules such as DNA, RNA, proteins and lipids and impair the plant functions (Table 3) (Foyer and Fletcher, 2001, Johnson *et al.*, 2003).

Table 6. Research scenario of anatomical characteristics in wheat under salinity.

Anatomical characteristic	Reference(s)
Root / Stem area	(Poljakoff-Mayber, 1975)
Epidermis thickness	(Al-maskri *et al*., 2014; Terletskaya *et al.*, 2015)
Epidermis cell area	(Hameed *et al.*, 2002;Terletskaya *et al.*, 2015)
Cortex thickness	(Akram *et al.*, 2001; Al-maskri *et al.*, 2014)
Cortex cell area	(Akram *et al.*, 2001; Al-maskri *et al.*, 2014)
Endodermis thickness	(Akram *et al.*, 2001; Al-maskri *et al.*, 2014)
Number of xylem vessels	(Akram *et al.*, 2001; Al-maskri *et al.*, 2014)
Xylem vessel area	(Akram *et al.*, 2001;Hameed *et al.*, 2002; Uga *et al.*,2008)
Phloem thickness	(Akram *et al.,* 2001; Hameed *et al.*, 2002; Al-maskri *et al.*, 2014)

Various solutes accretion including proline, glycine betaine, abscisic acid and polyol in the plant's cell are the requisite to retain the osmotic potential in vacuoles against the ions toxicity accumulated in cell compartments (khan *et al.*, 2009; Reis *et al.*, 2012). These are also known as compatible solutes or metabolites as these do not actually impede with the plants normal metabolic processes and protect the plants against salt stress (Sharma *et al.*, 1983). Kong *et al.* (2001) and Sairam *et al.* (2002) in their studies on wheat had determined the raise in solublesugars, proline, and glycine-betaine and abscisic acid contents under salt-stress induced cultivars in contrast to control cultivars.

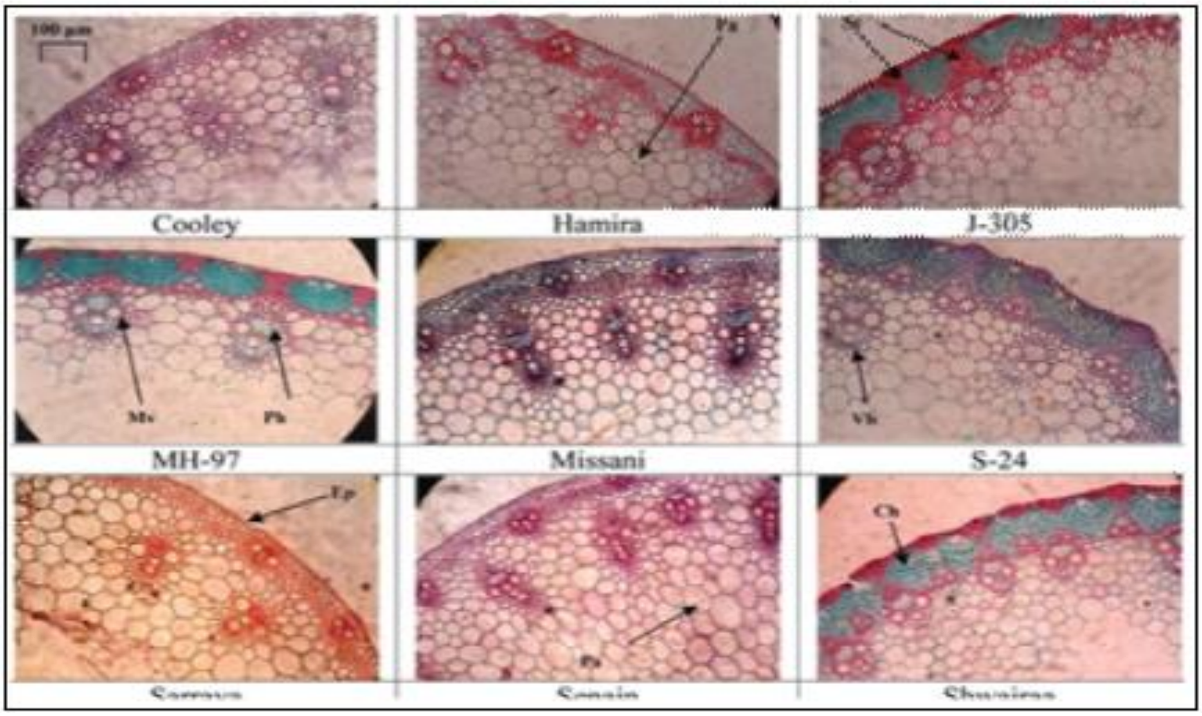

Fig. 1. Stem transverse sections of some wheat cultivars under salinity stress.

Note: Ep: Epidermis, Ch: Chlorenchyma, Pa: Parenchyma, Sc: Sclerenchyma, Mv: Metaxylem vessels, Ph: Phloem and Vb: Vascular bundle.

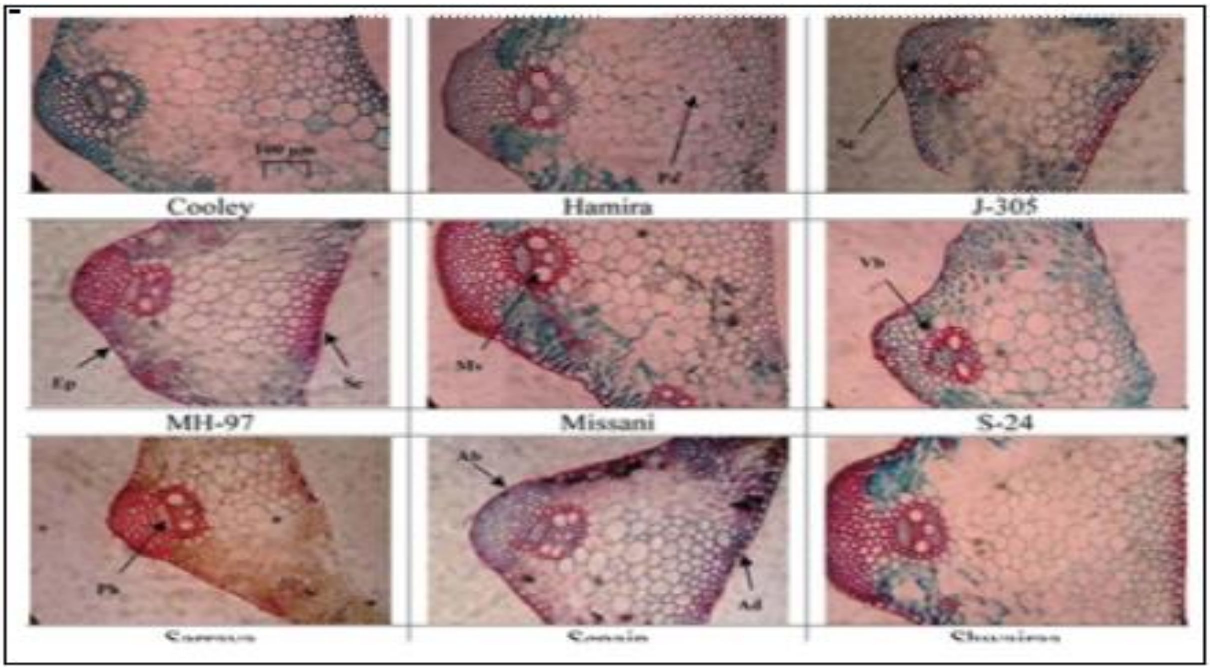

Fig. 2. Leaf midrib transverse sections of some wheat cultivars under salinity.

Note: Ab: Abaxial surface, Ad: Adaxial surface, Ep: Epidermis, Pa: Parenchyma, Sc: Sclerenchyma, Mv: Metaxylem vessels, Ph: Phloem and Vb: vascular bundle.

Literature available pointed out more chlorophyll and carotenoid contents in wheat under control condition with compared salt stressed plants (Sairam *et al.*, 2005; Khan *et al.*, 2009). This confirms the higher reduction in total chlorophyll as well as carotenoid contents in saline soils at all growth phases. Reduction in chlorophyll content has earlier been accounted in plants cultivated in saline environments and chlorophyll content has been recommended like one of the attributes for salinity-tolerant in plants (El-Samad *et al.*, 1993; Khatkar *et al.*, 2000; James *et al.*, 2002). Salinity stress enlarged the accumulation of Na^+ and Cl^- contents in wheat different parts especially in roots and leaves whereas reduced the K^+ and Ca^{2+} contents (Moustafa *et al.*, 1966; Gorham *et al.*, 1990; Rivelli *et al.*, 2002; Munns *et al.*, 2006).

Na^+ and Cl^- ions additions in plants provoke toxicity which has negative effects on vegetative and reproductive phases of growth.

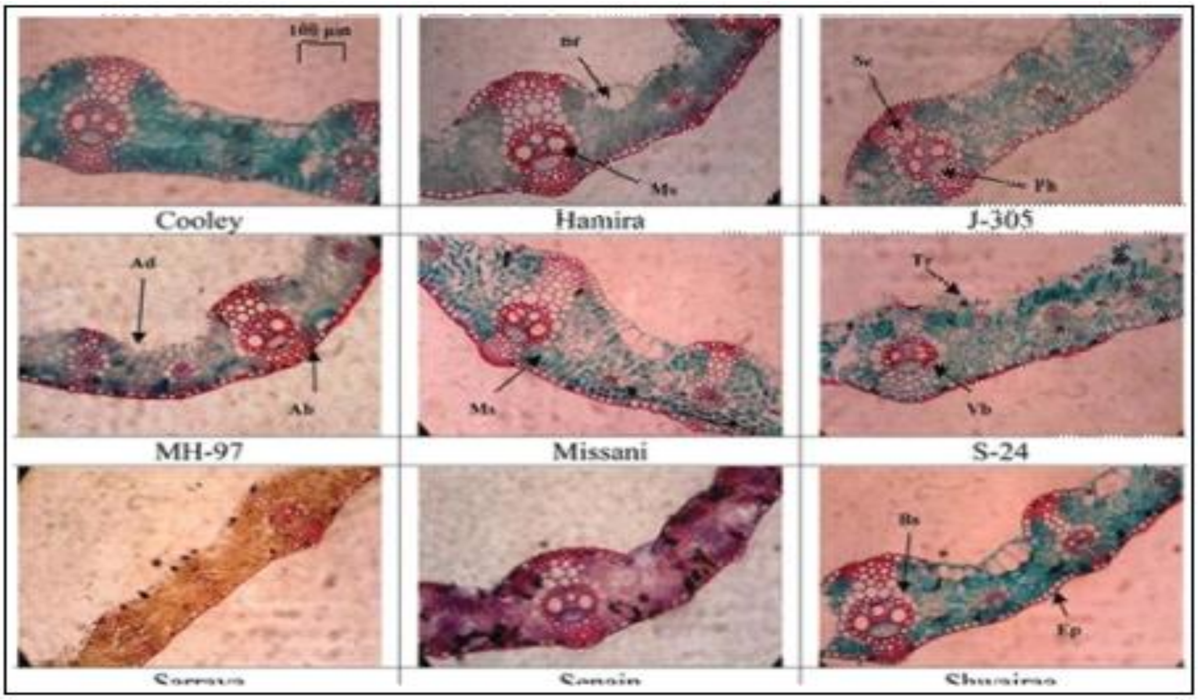

Fig. 3. Leaf lamina sections of some wheat cultivars under salinity stress.

Note: Ad: Adaxial surface, Ab: Abaxial surface, Bf: bulliform cells, Ep: Epidermis, Sc: Sclerenchyma, Ms: Mesophyll, Bs: Bundle sheath, Mv: Metaxylem vessels, Ph: Phloem and Vb: Vascular bundle.

Yield and yield components

Salinity stress limits the plant's growth and development which ultimately results in the poor productivity of agricultural crops (Mozafar *et al.*, 1986; Khan *et al.*, 2009; Daei *et al.*, 2009). It also harmfully affects on wheat crop and yield and yield components (Arfan *et al.*, 2007; Iqbal *et al.*, 2007).

Results revealed by Kingsbury *et el.* (1984), Bacilio *et al.* (2004) Ali *et al.* (2009) showed that spike length, spike diameter, number of tillers and straw yield were decreased whereas days to spike emergence were increased in different wheat cultivars (Table 5). Similarly, Goudarzi *et al.* (2008) and Khan *et al.* (2009) have reported decreased yield production in wheat cultivars in their studies.

In concise, extensive research has been made by researcher in respects of grains protein content, spike length, number of spikes, kernel weight and diameter, biological yield, grain yield, 1000 grains weight, seed number and grains number (Forster *et al.*, 1988; Acevedo *et al.*, 2002; Irfan *et al.*, 2005; Haque, 2006; Hina *et al.*, 2011, Sonia *et al.*, 2013; Hassan, 2015). Their results show that salinity exerts negative impacts on these yields attributes and consequently, productivity percentage is progressively decreased.

Molecular and genetical characteristics

There are extensive evidences present signifying that alteration in genes expression take place subsequent to salt stress experience in plants. Various genes have been recognized to be salinity stress influenced and formed special types of salinity stress associated proteins as well as enzymes (Ingram and Bartels, 1996; Bray, 1997; Shinozaki *et al*., 1998). Sal 1, Bnd 22, 25 kDa protein, P 150, 27 kDa protein, RAB21, vitronectin and fibronectin like some proteins and genes have been identified in different salinity-stress induced plants (Singh *et al*.,1987;Sadaka *et al*.,1991;Reviron *et al*.,1992;Benhayyim *et al*., 1993;Quintero *et al*.,1996). Moreover, dehydrins, vacuolar acid invertase, late embryo abundant proteins, ABA genes expression and proline induction have been too identified in salinity influenced plants (Close *et al.,* 1996; Pnueli *et al*., 2002; Trouverie *et al*., 2003; Anderson *et al*.,2004).

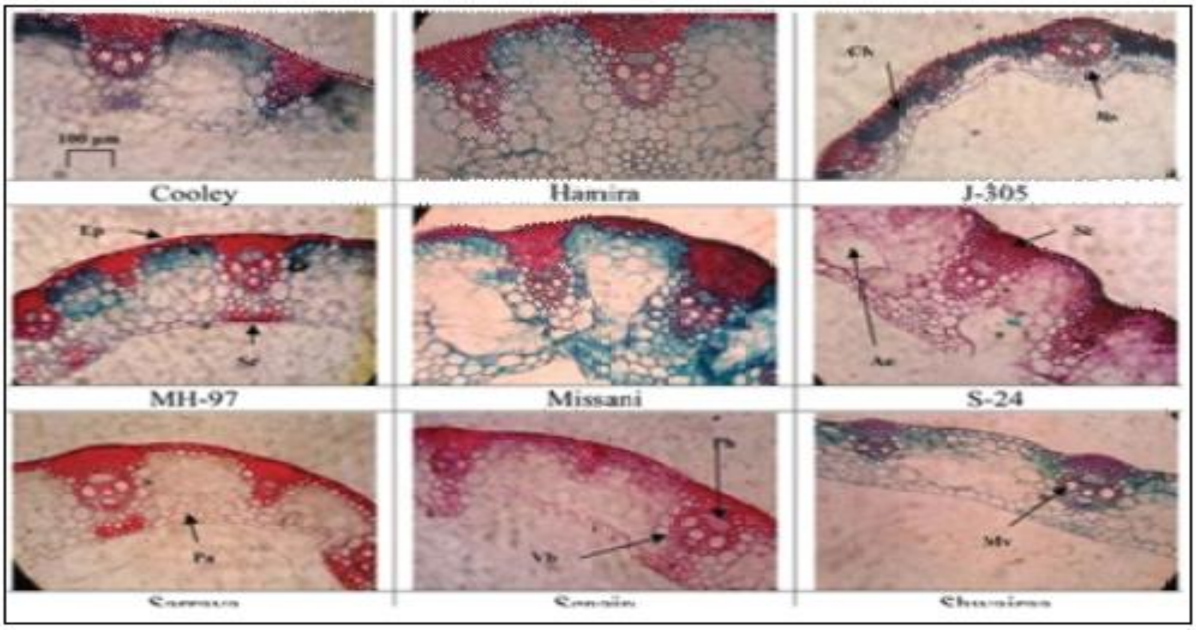

Fig. 4. Leaf sheath sections of some wheat cultivars under salinity stress.

Note: Ep: Epidermis, Ae: Aerenchyma, Bs: Bundle sheath, Pa: Parenchyma, Ch: Chlorenchyma, Sc: Sclerenchyma, Mv: Metaxylem vessels, Ph: Phloem and Vb: Vascular bundle.

Wheat responds to salt stress conditions with changing its genes expression and proteins production. Existing information on salt-responsive genes and proteins is yet limited because their functions have not been systematically determined. Wheat acclimation to salinity is regulated by abscisic acid (ABA) which enhances tolerance against salt shocks. The ABA stimulated acclimation is quick and corresponds with faster expression of the ESI (early salt induced) genes in the roots. Changes in 3A and 3D (Homologous chromosomes) to provoke stress-tolerance were also observed (Noaman *et al*., 2002).

Proline is an essential protein that performs very important function in salt stress tolerance. It is synthesized from pyrroline-5-carboxylate synthetase. During salt-stress, concentration of 26-kDa protein and proline content was observed significantly high in wheat which shows it is induced by salt-responsive genes to protect the plant during salinity (Elshintinawy and Elshourbagy, 2001). CDNA-encoding Cu/ZnSOD and Mn-SOD genes from wheat's chloroplast and mitochondrial were isolated and studied by Wu *et al*. (1999).

Their investigation showed that isolated Cu/Zn-SOD and Mn-SOD genes were salt stress inducible and play role in stress tolerance at different stages. Study reported by Temel*et al.* (2015) showed that elevated level of salt-stress in wheat suppressed the activities of both atls1 and α-tubulin genes inhibiting tubulin protein synthesis. In summarizing, elevated salt concentration brings changes at molecular and genetic level which retards the plants functioning properly (Table 4).

Anatomical characteristics

Salinity stress like other characteristics also affects on plant's root, leaf and stem attributes (Poljakoff-Mayber, 1975; Hameed *et al.*, 2010). Root is the first organ which experiences the earliest salinity stress (Solomon *et al.*, 1986; Terletskaya *et al.*, 2015). Relationship of wheat's anatomical traits showing disparity in tolerance against the salinity stress is essential to identify the salinity influences at all levels (cell, tissue and organ) (Terletskaya *et al.*, 2011).

Salinity imposition in wheat causes alterations at cell as well as tissue levels which results in the decrease of overall cells dimension of root, stem and leaf parts (Terletskaya *et al.*, 2017). Akram *et al.* (2001) in their study reported different structural variations such as enhanced succulence, changes in size and number of stomata, cuticle thickness, earlier lignifications and changes in number and diameter of xylem vessels of wheat under salt-stressed conditions. Akram *et al.* (2002) reported a decrease in the leaf dermal cells expansion in wheat grown under salt stress. Hameed *et al.* (2002) and Al-maskri *et al.* (2014) reported that as anatomical characters, both leaf and leaf in wheat were greatly affected. The dermal tissues showed considerable decrease in epidermal cell thickness and area, stomatal area and increased number of stomata. Similarly, there were variations observed among ground and vascular tissues which showed a significant reduction in cortex cell area and interveinal space, vascular bundle area and metaxylem vessels area (Table 6, Fig. 1-4, Source: Al-maskri, *et al.*,2014). The tendency of reduction of xylem vessel area by increasing salt stress was differing to the trend in vascular bundle area, and is in concurrence with Uga *et al.* (2008) whose work hints that vascular bundle area and xylem vessels thickness or area are in separate genetic regulation .Smaller xylem vessels thickness or area has been accounted earlier in rice roots with increase osmotic stress (Yambao *et al.*, 2003; Mostajeran and Rahimi-Eichi, 2008). Therefore, xylem cells with smaller and narrow vessels are physiologically more confined against embolisms or cavitation (Rury and Dickinson, 1984). Due to narrow xylem vessels, risk of cavitation is reduced resulting in better water uptake.

Conclusion

Salinity is a threatening abiotic stress limiting normal growth and development of wheat reap which ultimately results in low productivity. Extensive study has been performed to investigate the salinity impacts on wheat plants to improve growth and productivity. Soil salinity detrimentally affects the various characteristics of wheat plants including germination rate, early seedlings growth, plant height, root length, number of leaves, leaf area, fresh and dry weight, water relations, stomatal conductance, chlorophyll content, rate of photosynthesis and transpiration and ionic relations and makes complicated to study all aspects at same moments.

Suggestion

Over the previous few decades, research carried out on wheatplants put lights on various sides of the biochemical and molecular processes triggered during salt-stress. But, lots of assessments and investigations still recline ahead prior to productively improving wheat growth and development in saline conditions.Therefore, it is need of the hour for some combined approaches to accelerate the detection and categorization of specific proteins and genes concerned in salt-tolerance that can assist to produce the best wheat varieties by modified molecular markers.

Acknowledgement

The authors are thankful to Assistant Professors, Dr .Nargis Naz and Dr. Sadaf Zehra (Department of Life Sciences, The Islamia University of Bahawalpur) for their inspiration and moral support during manuscript preparation.

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