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PHYSIOLOGICAL IMPLICATION OF MORINGA EXTRACTS APPLICATIONS FOR OSMOLYTES PRODUCTION IN MAIZE CROP UNDER HEAT STRESS

ASIMA BATOOL[1], ABDUL WAHID[2], GHULAM ABBAS[3*], MUHAMMAD NAEEM AKHTAR[4], ZUHAIR HASNAIN[5] AND NASIRA PERVEEN[6,]

[1]Department of Botany, University of Agriculture Faisalabad, Pakistan,
[2]Department of Botany, University of Agriculture Faisalabad, Pakistan
[3]Department of PBG, UAF Subcampus Okara Pakistan
[4]Pesticide Quality Control Lab, Multan, Pakistan
[5]PMAS Arid Agriculture University Rawalpindi, Pakistan
[6]Aridzone Agricultural Research Institute, Bhakkar, Pakistan
[*]Corresponding author's Email: abbas1434@yahoo.com

Abstract

The research work presented in this manuscript was carried out to know the pattern of various osmolytes accumulation in maize plants exposed to heat stress after application of different types and modes of extracts of moringa plants.

Two maize hybrids (SB-11 heat tolerant, ICI-984 heat sensitive) were grown under control and heat stress conditions. These were exposed to exogenously application of water and aqueous leaf (fresh and shade dried) and flower extracts of moringa plant using three different modes i.e., seed priming, medium supplementation and foliar spray.

Data suggested that all the types and modes of moringa extract applications were substantially effectual in enhancing the osmolytes synthesis. Maize plants from heat tolerant hybrid indicated a greater while heat stressed plants revealed a lesser accumulation of all the omsolytes. Among the modes of extract application, the medium supplementation of extracts was substantially more effectual than foliar spray while seed priming was the least effectual. Moreover, among the osmolytes, the most conspicuous accumulation was recorded for shoot free proline and shoot glycinebetaine, while total free amino acids did not display much variation across the application modes.

The differences in the synthesis of osmolytes may be attributable to the possible differences in the phytochemical constituents of extracts. Such a biosynthesis pattern of osmolytes may lead to heat tolerance in maize hybrids.

Keywords: Maize, Heat stress, Proline, Glycinebetaine, Moringa, Seed priming, Foliar spray, Medium application.

Introduction

The research on the interaction between abiotic stresses crop plant species has gained considerable attention and significant progress has been achieved in this field. However, promising crop germplasm is being regularly introduced with new genetic makeup and the determination of responses of these materials to abiotic and other stresses is a demanding field of research. Recent studies have shown that considerable inherent potential for abiotic stress resistance (Wahid, 2007, 2008b and Mahmood *et al*., 2012).

In general, plants have natural competency to resist environmental stresses, additionally, the tolerance in plants can be improved by exogenious application of growth promoting hormones or chemicals at appropriate concentrations. A large number of available studies show that this approach is viable and beneficial in enhancing crop yields significantly (Gill & Tuteja, 2010; Yasmeen *et al*., 2013; Abdalla, 2013). More commonly seed priming and foliar spray and rarely medium supplementation are the modes adopted to apply these chemicals. Despite all the yield benefits, this approach may not be economically much viable due to cost-intensiveness of the chemicals. Recently there is a trend towards the exogenous use of plant extracts as a natural resource for improvement in growth of crop and its yield in standard or subversive growth condition (Siddhuraju & Becker, 2003; Yasmeen *et al*., 2014). Few research studies revealed that use of plant extracts, which contains different primary and secondary plant products, can be a cheap and cost-effectual approach to enhance the growth and yield of economically important crop plants.

Among the few plants used for applying their extracts to enhance the growth and outcome, moringa has been relatively more investigated because, when extracted it provides growth enhancing substances such as hormones, vitamins, antioxidants and minerals, which are essentially needed for better growth of plants and development too (Ferreira *et al*., 2008; Ilyas *et al*., 2015, Batool *et al*., 2019). Moringa is a commonly growing tree species and is now more intensely grown due quite a few health benefits to mankind. Its flowers are used as vegetable and roots are used to make pickle in the human diet. It has a lot of therapeutic and medical effects for humans (Anwar *et al*., 2007; Varmani & Garg, 2014). The available studies show that externally applied aqueous moringa leaf extract on the plants like wheat, tomato etc. improved the antioxidant potential of these plant species (Yasmeen *et al*., 2013). However, the comparative impacts of moringa plant's aqeous extract of several parts, when applied via different methods in improving crop growth has been rarely investigated under control or heat stress has been rarely investigated.

An amphoteric quaternary amine, Glycinebetaine (GB), a compatible solute help plants to escape number of stresses, such as heat and salt stresses (Sakamoto and Murata, 2002). The production of GB depends on extent of stress, duration and plant species (Ashraf & Foolad, 2007). For example, Quan *et al*., (2004) reported greater glycinebetaineamassing in maize under drought stress. Wahid & Close (2007) observed GB accumulation in sugarcane under heat stress. Some plant species like tobacco (*Nicotianatabacum*) mustard (*Brassica compestris*), Arabidopsis (*Arabidopsis thaliana*) and (*Oryza sativa*), unable to produce glycinebetaine but through genetic engineering it make possible to incorporate glycinebetaine biosynthetic pathways (Quan *et al*., 2004).

In higher plants, proline has been recognized to exist largely and generally amass in bulk quantity with under different abiotic stresse (Kavi-Kishore *et al*., 2005; Ashraf & Foolad, 2007). Accumulation of soluble sugars under heat stress has been described in sugarcane, which have great implications for cell water maintenance in high temperature (Wahid & Close, 2007). The tomato plants failed to fruit set due to distraction of sugars metabolism and transport of proline during the development of male reproductive organs in high temperature (Sato *et al.,* 2006).

Thus, analyzing the scarcity of information in this regard, the proposed research was carried out to find-out the usefulness of extracts from various parts of moringa plants by different application modes in enhancing heat tolerance in maize through accumulation of metabolites like proline, glycine betaine and fatty acid contents.

Materials and Methods

To examine the interaction of exogenous application of the optimized levels of all the extracts on maize hybrids under control and heat stress conditions, three experiments i.e., seed priming; medium supplementation and foliar spray were done separately. In each of these experiments, seeds of two maize hybrids SB-11 (heat tolerant) and ICI-984 (heat sensitive) were sown in plastic pots, consisting of10 kg of washed river sand and plants were applied/ replaced with half strength nutrient solution (Yoshida *et al*., 1976) on five day interval. Newly emerging leaves of moringa obtained from young fully grown trees of moringa were selected for this study performed in Department of Botany, University of Agriculture, Faisalabad. As per method described by Price (2007), the fresh Moringa leaf extracts (MLE) were prepared.

For seed priming experiment, the seeds of selected hybrids were soaked in 3, 5 and 5% dilution of the concentrated extracts of MFLE, MDLE and MFE, respectively and sown in post containing sand and grown under ambient conditions. For medium supplementation experiment, 10 day old plants were applied with 3, 10 and 10% dilution of the concentrated extracts of MFLE, MDLE and MFE, respectively. For foliar spray, 10 day old plants were sprayed on the leaves with 3, 5 and 15% dilution (added with Tween-80 as surfactant at the rate of 10%) of the concentrated extracts of MFLE, MDLE and MFE, respectively with a manual sprayer.

After treatment application, single set of 10 days older plant from each experiment was kept in the net house while other set was kept in the plexi-glass fitted canopies for heat stress (7-10°C) higher than ambient temperature. The plants from all the experiments were grown for 10 days and then harvestedsamplesoffresh shoot and root were preserved at -80°C till analysis. These preserved samples were used for the determination of gross free amino acids, glycine betaine and proline. GrossFree Amino Acids: Gross free amino acids were determined according to Hamilton and Van Slyke (1943). Free Proline was measured following the method of Bates *et al*., (1973). In the same context, the procedure developed by Grieve and Grattan, 1983 was used for determination of Glycinebetaine.

All three experiments were planned in completely randomized (CR) design in triplicated arrangement. Analyses of variance for all attributes were done using Statistix 8.1sttistical software and means were subjected to Duncan's Multiple Range Test for determining the variation among various factors and their interactions separately (Steel *et al*., 1996).

Results

Shoot gross free amino acids (TFA): The results showed that in both hybrids, seed priming mode of extract application was effectual in enhancing shoot TFAunder either condition. Overall, under controlledenvironment, ~11% upturn in the shoot TFAin maize hybrid SB-11 and ~12% increase in shoot TFA in in maize hybrid ICI-984 was noticed. However, under heat stress there was about 13% enhancement of shoot TFA in maize hybrid SB-11 and 10% in maize hybrid ICI-984 over the respective control condition. Among the various priming treatments, MDLE were the most effectual in both maize hybrid SB-11 and ICI-984 under control or in high temperature (Fig. 1a).

Both hybrids have shown positive response according to data on medium supplementation of extract; although hybrid SB-11 better response than ICI-984 both under controlled as well as underhigh heat condition with regard to shoot TFA. Under control condition, there was a maximum of 33 and 15% upturn in shoot TFA in SB-11 and ICI-984, respectively with MDLE. Applied heat stress increased the shoot TFA in non-supplemented plants SB-11 (by ~4%) but reduced in ICI-984 (by 12%), the extracts application increased it in both the hybrids maximally with MDLE which was 37% in in maize hybrid SB-11 and 33% in in maize hybrid ICI-984. Overall, among the various extracts supplemental treatments, MDLE was the most effectualand then MLFE under control and MFE in high temperature in both the hybrids (Fig. 1b).

From the results of foliar spray of extracts it was noted that the shoot TFA got increased in both maize hybrids in both optimum and high temperature conditions, although response of in maize hybrid SB-11 was better than in maize hybrid ICI-984. Under control condition there was maximumupturn of 46% TFA while in in maize hybrid ICI-984, an increase was 36% with the foliar spray of MDLE. Applied heat stress although further improved shoot TFA in the unsprayed plants of both the hybrids, the extract spray was more effectual in enhancing this attribute in SB-11 by 35% and in ICI-984 by ~31% Among the various foliar sprays, MDLE was the most effectual (Fig. 1c).

With significant differences in the extracts application modes, overall soil application was the most effectual followed by foliar spray in increasing shoot TFA in both hybrids (Fig. 1).

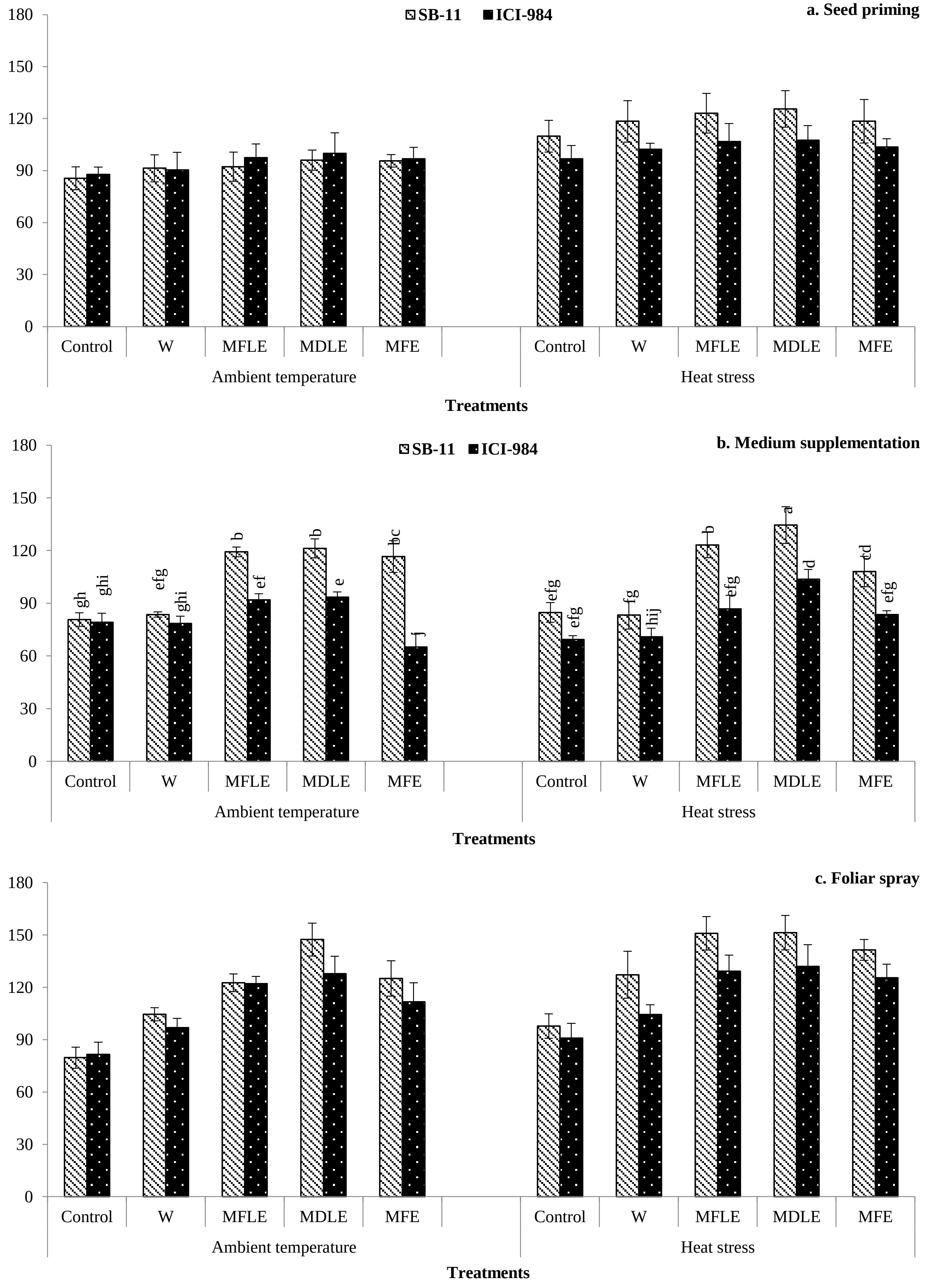

Fig. 1. Changes in total free amino acids of differentially heat responsive maize hybrids to the external application of water treatment, MFLE, MDLE and MFE subject to heat stress in seed priming, medium supplement and foliar spray application modes.

Root total free amino acids (TFA): As regards root TFA, the results showed that seed priming with moringa parts extracts enhanced the root TFA in both the maize hybrids. Root TFA indicated development under both conditions, but it was much greater under control than under heat stress. Under controlled environment, the increase in TFA was ~32% in maize hybrid SB-11 and 23% in hybrid ICI-984. Under heat stress, however, this improvement was 6% with MFLE and MDLE in SB-11 while only 3% with MFLE in ICI-984. Among the various priming treatments, MFLE and MDLE were the most effectual for SB-11 and MFLE for ICI-984 in improving this attribute (Fig. 2a).

In case of medium supplementation of extract, data revealed that both hybrids responded in different ways to the extracts as well as control and heat stress treatments, although hybrid SB-11 exhibited more improvement than ICI-984. Under control condition, SB-11 manifested maximum of 32% while ICI-984 expressed 27% upturn in root TFA through MDLE. Exposure to heat stress increased the root TFA in the non-supplemented plants of both the hybrids, the application of extracts was effectual in improving the root TFA by 14% in SB-11 while 19% in ICI-984 with medium supply of MDLE. Among the various priming treatments, MDLE was the most effectual followed by MFLE and MFE especially under heat stress (Fig. 2b).

Data regarding foliar spray of moringa extracts showed that both the hybrids showed an improvement in in root TFA both under control and heat stress, although hybrid SB-11 expressed more improvement than hybridICI-984. Around 36% increase was found in hybrid SB-11 while just 19% increase in root TFA by hybrid ICI-984under controlled treatment. Although applied heat stress improved root TFA in the unsprayed plants of both the hybrids, MDLE spray was able to maximally improve it by 22% in SB-11 and by 10% in ICI-984. Among the various priming treatments, MDLE was the most effectual in improving root TFA followed by MFLE (Fig. 2c).

Overall there were significant differences in the extracts application modes, the extracts types, hybrids and heat stress conditions, nonetheless medium supplementation of extract was the most effectual followed by foliar spray in improving root TFA in both the hybrids (Fig. 2).

Shoot free proline: The data revealed that all the seed priming treatments were effectual in enhancing the shoot free proline contents in both the hybrids under normal as well as under stress treatment, although both maize hybrids showed varying response. Under control treatment, there was an uplift of 21% in shoot free proline with different treatments in both the hybrids over untreated controls. However, under heat stress the shoot free proline enriched by ~32% in maize hybrid SB-11 and ~19% in hybrid ICI-984 through MDLE. Thus among the various priming treatments, MDLE were the most effectual followed by MFLE and MFE (Fig. 3a).

The medium supplementation mode of extract application was effectual in improving the shoot free proline contents in both the hybrids to a similar extent (~27-28%) especially with MDLE under control condition. However, under heat stress non-supplemented plants of both the hybrids although improved shoot free proline, it was further improved by ~37% in maize hybrid SB-11 and 33% in maize hybrid ICI-984. All the priming treatments improved this attribute in both the maize hybrids under control treatment or heat stress treatment, but MDLE treatment was the most efficient followed by MFE (Fig. 3b).

The foliar spray of extract was helpful in improving the shoot free proline contents in both the hybrids to a relatively greater extent in hybrid SB-11 (~26%) than in hybrid ICI-984 (24%) with MDLE under control treatment. Under heat stress, however, non-sprayed plants of both the hybrids improved shoot free proline, it was further improved by ~37% in maize hybrid SB-11 and 25% in maize hybrid ICI-984. All the priming treatments improved this attribute in both the hybrids under control treatment or heat stress treatment, but MDLE was the most effectual followed by MFLE (Fig. 3c).

Overall there were great differences in the hybrids, prevailing growth conditions and type of extracts applied exogenously but SB-11 indicated a greater improvement; MDLE was the most effectual extract type medium supplementation was the most efficient mode of extract application in improving shoot free proline (Fig. 3).

Root free proline: It was revealed from the data that all the seed priming strategies successfully enhanced the root free proline in both maize hybrids although; the degree of improvement was quite varying under both the conditions. Under control condition, there was maximally 12% and 10% increase in root free proline in hybrid SB-11 and maize hybrid ICI-984 respectively for MDLE. However, under heat stress treatment, MDLE pretreatment was highly effectual in improving this parameter in ~25% in SB-11 and ~20% in ICI-984. Among the various priming treatments, MDLE followed by MFE were the most effectual in enhancing this parameter (Fig. 4a).

Medium supplementation of moringa extract revealed that both the maize hybrids responded differently to the extracts and applied heat stress treatments, whilst the response of SB-11 was better than ICI-984 under either condition. Under control treatment, the root free proline improved by 46 and 44% in SB-11 and ICI-984 respectively and that with MDLE supplementation. Applied heat stress although markedly improved the root free proline synthesis in both the hybrids, the application of extracts was quite effectual in further improving its contents although greatly in hybrid SB-11 (42%) and slowly in hybrid ICI-984 (17%) using MDLE. Among the medium supplements, treatment of MDLE was the most proficient followed by treatments of MFLE and MFE (Fig. 4b).

As regards foliar spray of extracts, it was noticed that under control condition both the hybrids responded differently to the extracts and applied heat stress, although SB-11 responded relatively better with MFLE spray (30%) while ICI-984 with MDLE spray (29%). Applied heat although improved root free proline in non-sprayed plants of both maize hybrids, it was additional increase using extracts sprayedapproximately ~28% in maize hybrid SB-11 and by 16% in maize hybrid ICI-984 through MDLE. All kind of foliar spray treatments improved this attribute in both the hybrids; however, MDLE was the most efficient followed by treatment MFLE and MFE (Fig. 4c).

Overall there were great differences in the hybrids, prevailing growth conditions and type of extracts applied exogenously but SB-11 indicated a greater improvement; MDLE was better improviser extract, while medium supplementation mode was the most efficient mode of extract application in improving root free proline (Fig. 4).

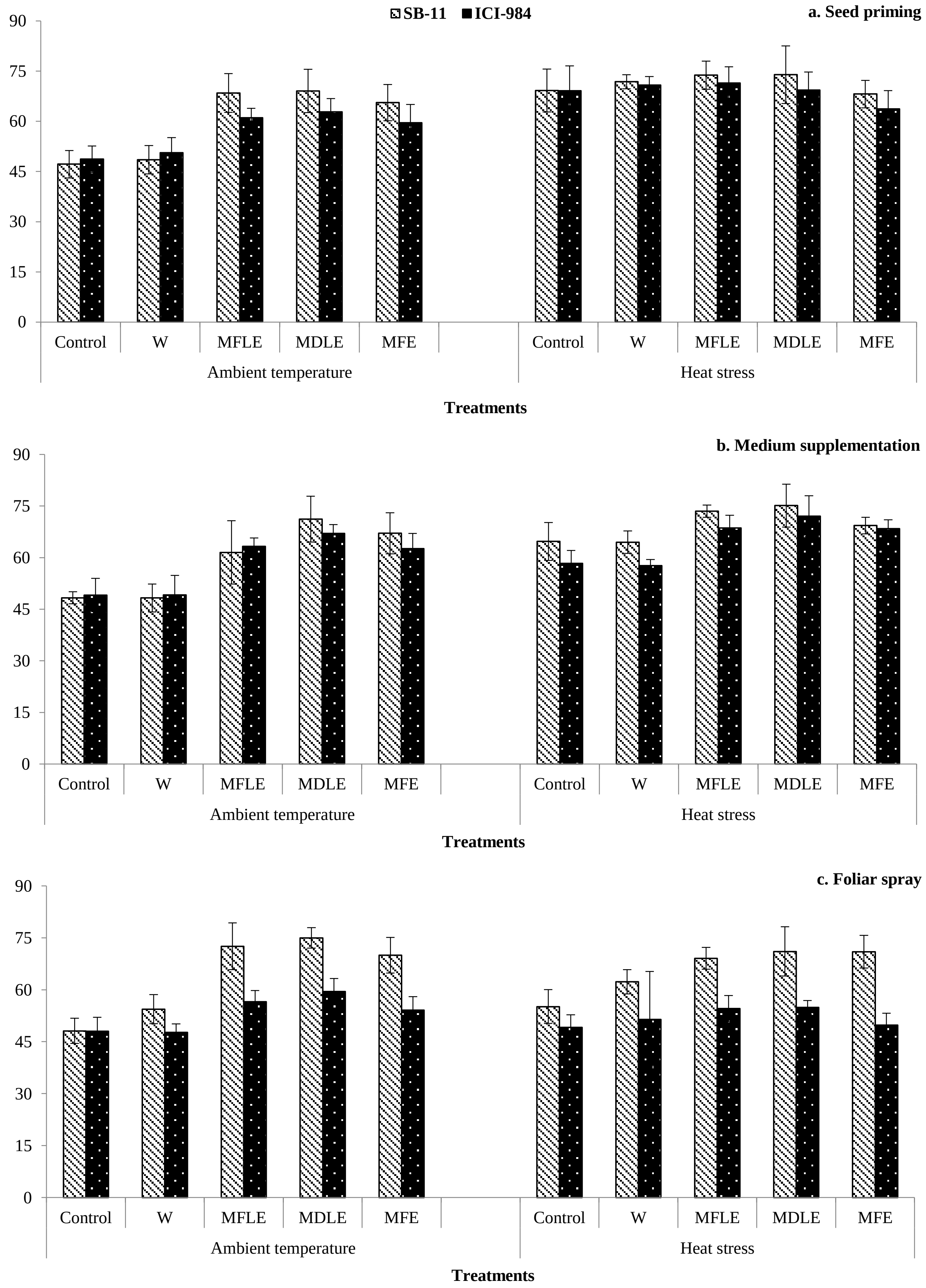

Fig. 2. Changes in root total free amino acids of differentially heat responsive maize hybrids to the external application of water treatment, MFLE, MDLE and MFE subject to heat stress in seed priming, medium supplement and foliar spray application modes.

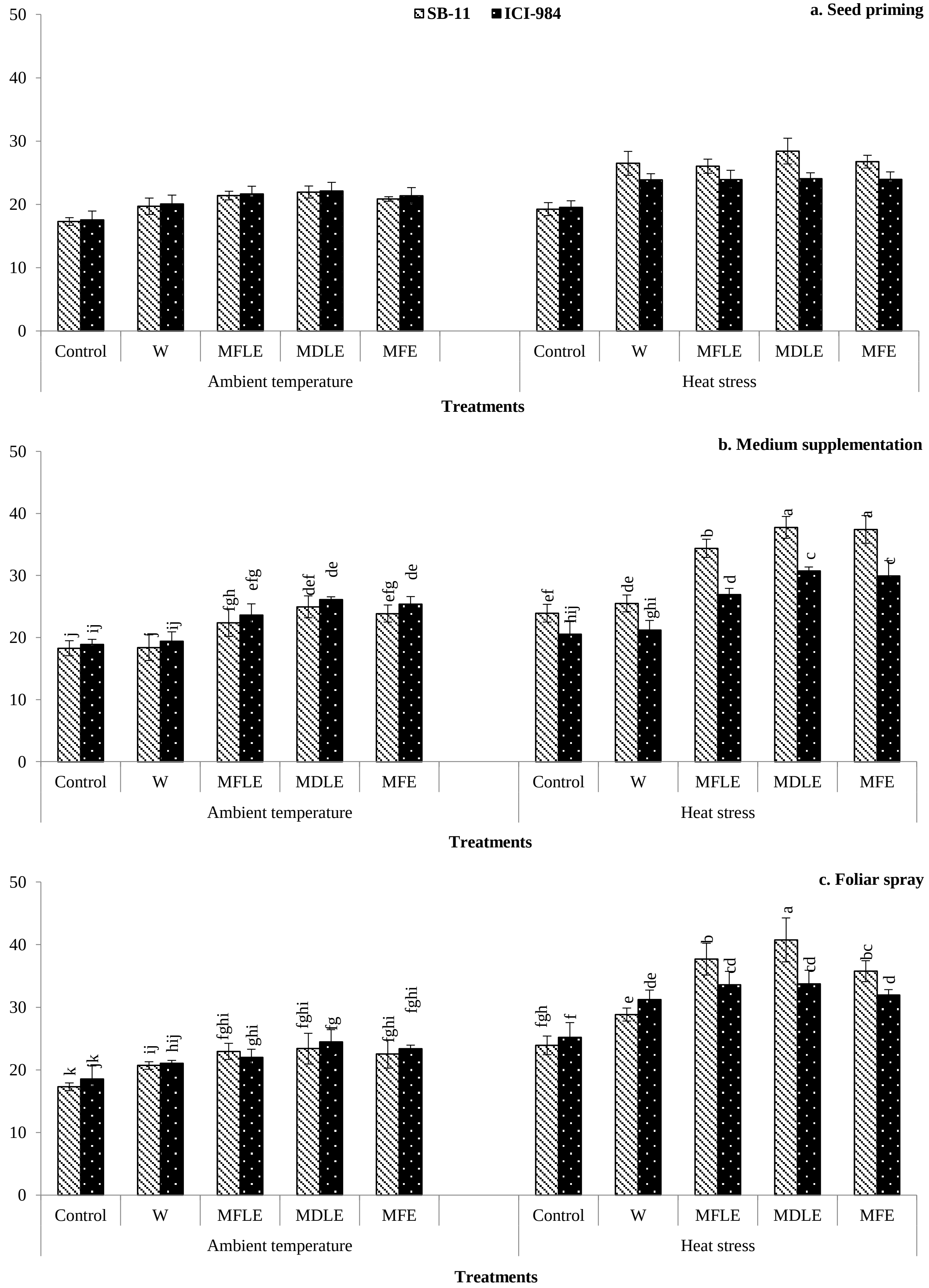

Fig. 3. Changes in shoot free proline of differentially heat responsive maize hybrids to the external application of water treatment, MFLE, MDLE and MFE subject to heat stress in seed priming, medium supplement and foliar spray application modes.

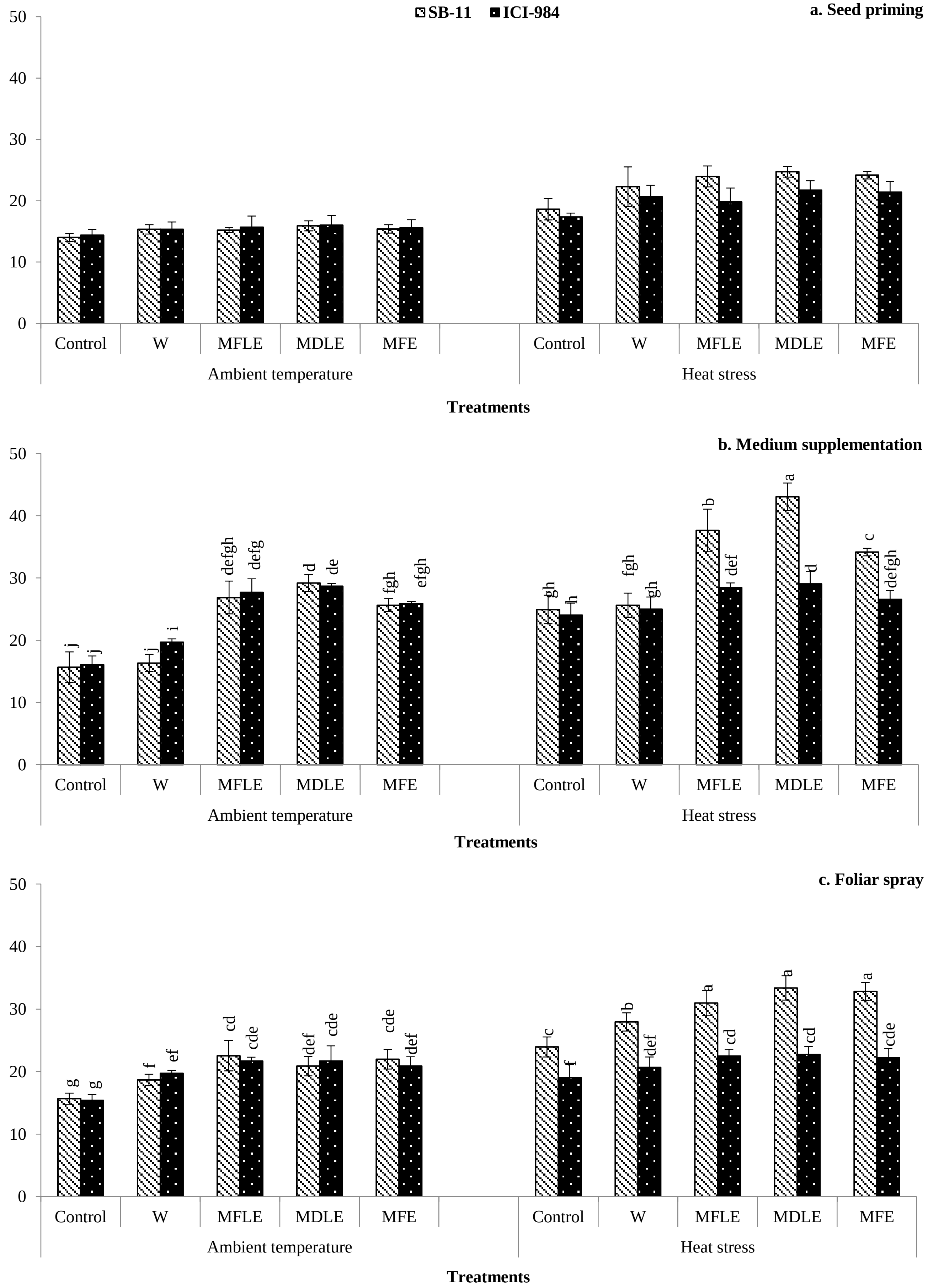

Fig. 4. Changes in root free proline of differentially heat responsive maize hybrids to the external application of water treatment, MFLE, MDLE and MFE subject to heat stress in seed priming, medium supplement and foliar spray application modes.

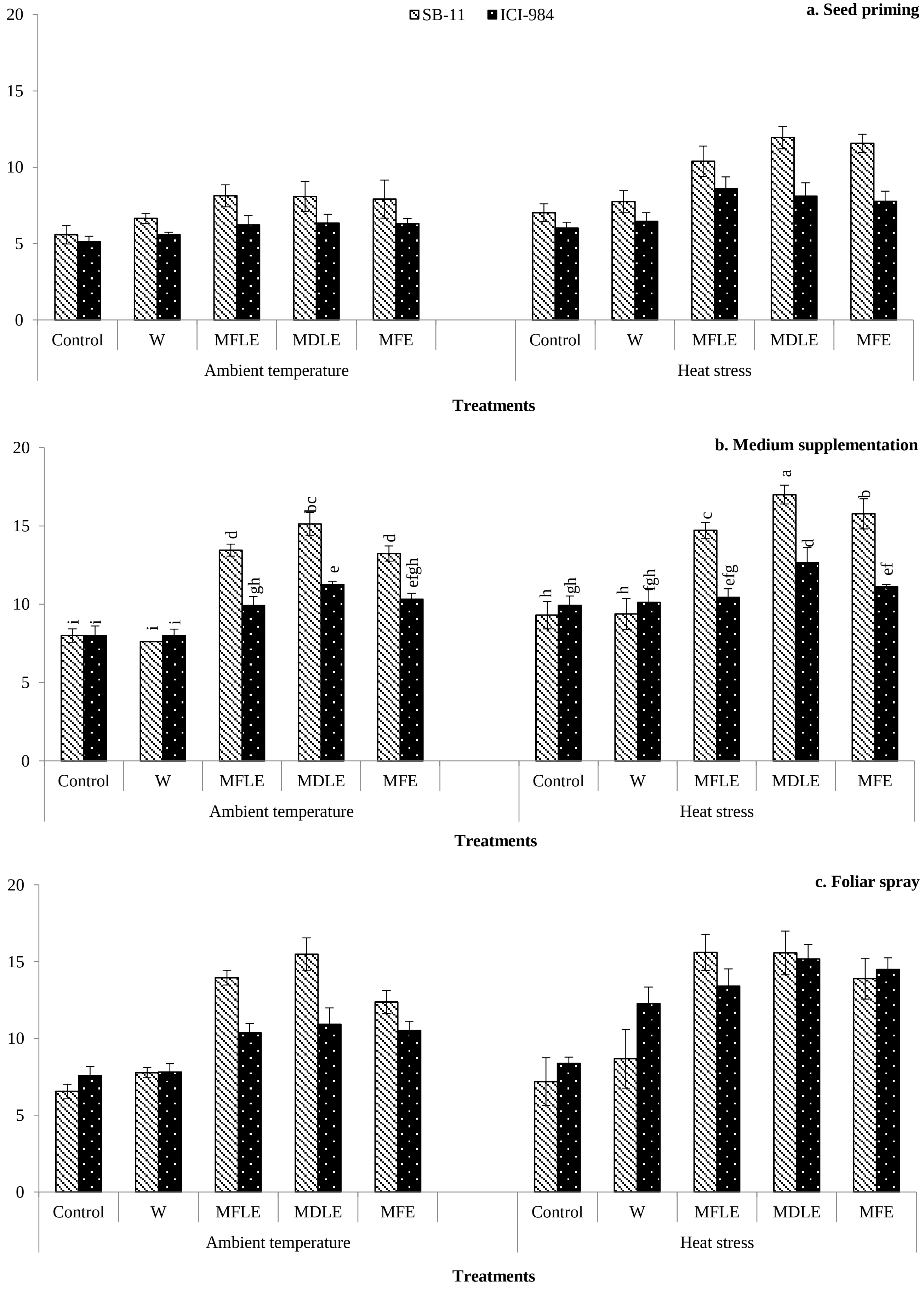

Fig. 5. Changes in shoot glycinebetaine of differentially heat responsive maize hybrids to the external application of water treatment, MFLE, MDLE and MFE subject to heat stress in seed priming, medium supplement and foliar spray application modes.

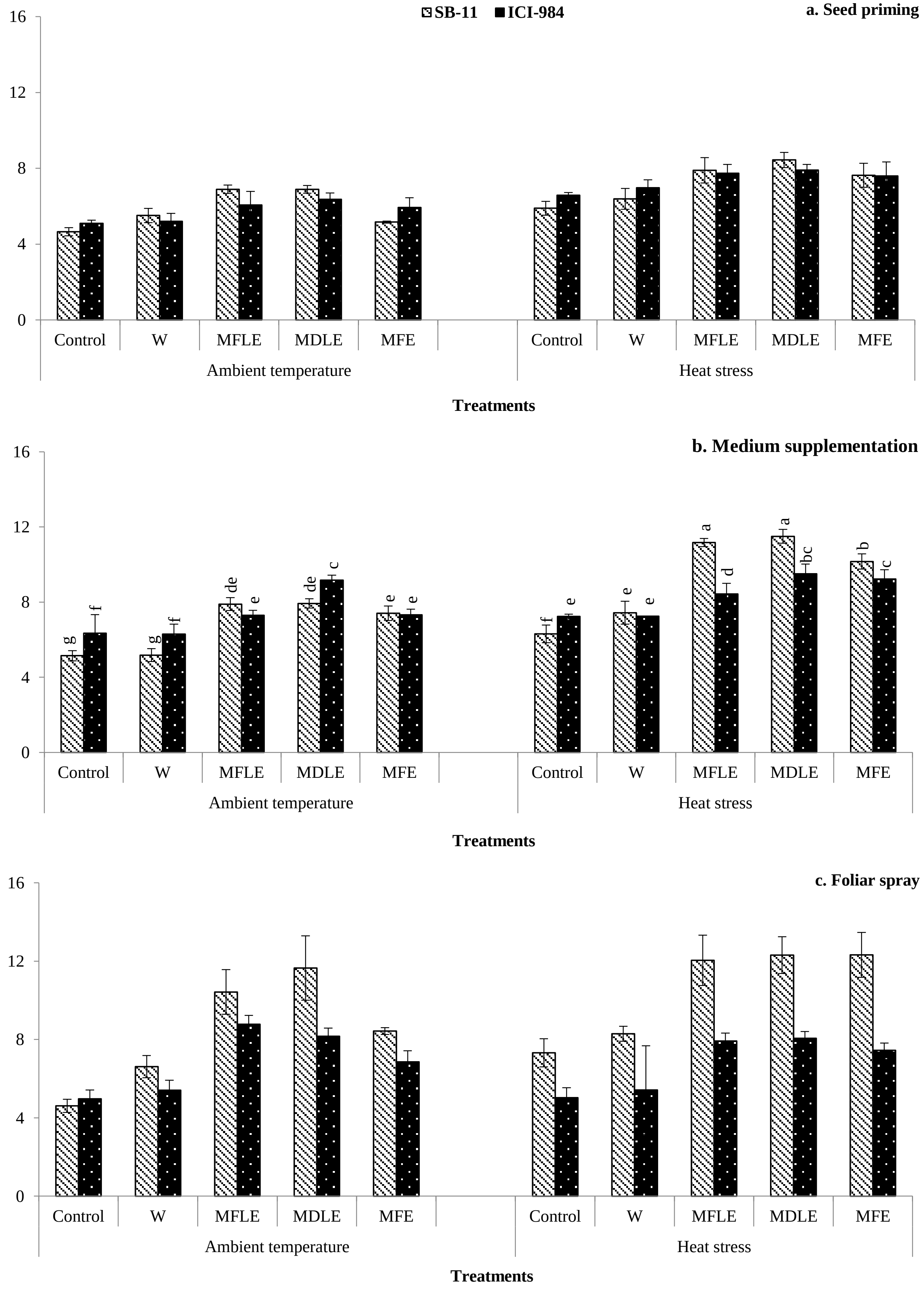

Fig. 6. Changes in root glycinebetaine of differentially heat responsive maize hybrids to the external application of water treatment, MFLE, MDLE and MFE subject to heat stress in seed priming, medium supplement and foliar spray application modes.

Shoot glycinebetaine (GB): As revealed from data, seed priming with extracts or water enhanced the shoot GB in both maize hybrids and under both control and high heat environment. Under control treatment condition, there was ~31% uplift in shoot Glycinebetaine in maize hybrid SB-11 (with MFLE) while only 19% increase in maize hybrid ICI-984 (with MDLE). However, under heat stress this improvement was much greater being 41% in SB-11 (with MFLE) and 31% in ICI-984 (with MDLE). Among the various priming treatments, MDLE was the most effectual followed by MFE under either condition (Fig. 5a).

Medium supplementation of extract data showed that both the hybrids responded well to the extracts under both control and heat stress, although SB-11 responded much better than ICI-984. Under control condition SB-11 indicated 47% while hybrid ICI-984 displayed 29% enhancement in shoot GB with moringa medium application of MDLE in both. Applied heat stress although improved the shoot GB in non-supplemented plants of both the hybrids, the application of extracts further improved this attribute by 45% in maize hybrid SB-11 and by 21% in maizehybrid ICI-984 with MDLE again. Among various types of medium supplements, MDLE were the most effectual followed by MFLE (Fig. 5b).

As regards foliar spray of extracts revealed that under control condition both the hybrids showed varying behavior but effectively to the extracts and heat stress treatments, although hybrid SB-11 responded more increase (58%) than hybrid ICI-984 (29%) through MDLE spray. Applied heat although improved shoot GB in non-sprayed plants of both the hybrids, it was additionalenhanced with moringa extracts spray by ~54% in hybrid SB-11 through MFLE and MDLE and also by 45% in hybridICI-984 using MDLE. Every treatment of foliar spray enriched this feature in both maize hybrids; however, MDLE was the most efficienttrailed by MFLE and MFE (Fig. 5c).

Overall there were great differences in the type of extracts applied; prevailing growth conditions and hybrids but SB-11 indicated a greater improvement. MDLE was better extract, while medium supplementation was highly effectual in improving shoot GB (Fig. 5).

Root glycinebetaine (GB): Data revealed that seed priming mode of extract application increases the root GB under controlled or high temperature conditions in both the hybrids. Under controlled environment, overall ~32 upturn in root Glycinebetaine in hybrid SB-11 (with MFLE and MDLE) and 20% enhancement in hybrid ICI-984 (with MDLE) was found. Under heat stress condition, however, priming with MDLE was beneficial for both the hybrids since there was an development in this factor by 30% in SB-11 and by 17% in ICI-984. Among the various priming extracts source wise, MDLE was most effective followed by MFLE & MFE over their respective controls (Fig. 6a).

For medium supplementation of extract, results revealed that both the hybrids showed varying response but effectively to the moringa extracts and applied control & heat stress treatments, although maize hybrid SB-11 responded more positively thanhybrid ICI-984 for root Glycinebetaine. Under controlledstate, hybrid SB-11 presented a maximum of 35% improvement with MFLE and MDLE while ICI-984 manifested 31% increase with MDLE. Exposure to heat stress although increased the root GB in the non-supplemented plants of both the hybrids, the application of extracts was effectual in furthering the root GB by 45% in hybrid SB-11 while 24% in hybrid ICI-984 using medium supply of MDLE. Among medium supplementation treatments, MDLE was the most effectivetrailed by treatment MFLE & MFE especially under heat stress condition (Fig. 6b).

Data regarding foliar spray of moringa extracts showed that both the hybrids got improved in root GB both under control and heat stress, although SB-11 indicated a much higher improvement than ICI-984. Under control condition ~60% improvement was shown by SB-11 while 39% by ICI-984 with MDLE. Although applied heat stress improved root GB in the unsprayed plants of both the hybrids, MDLE spray was able to maximally improve it by 41% in maize hybrid SB-11 with MFE and by 38% in hybrid ICI-984 through MDLE. Among various moringa foliar sprays, MDLE was the most effectualin improving root GB followed by MFE (Fig. 6c).

Overall there were significant differences in the moringa extracts application modes, maize hybrids and temperature / heat stress conditions, types of moringa extracts, neverthelessmoringa extract applied via medium supplimentation was highly effective trailed by foliar spray in improving root Glycinebetaine in both maize hybrids (Fig. 6).

Discussion

The accumulation of osmolytes at much reduced amounts in free form is necessary for plant's normal growth and development to maintain the osmotic balance. However, their accumulation pattern and role becomes much more pivotal, since they maintain the cell water balance under abiotic stress conditions (Taiz *et al*., 2015). External application of stress reducing compounds is quite often made to enhance the tolerance of plants against stress, by maintaining the cell water balance (Hasanuzzaman *et al*., 2013). As regards use of moringa leaf, Yasmeen *et al*., (2013, 2014) successfully improved the wheat and tomato water relations with the 3% use of MLE under normal or salinity stress conditions.

In the current research, two maize hybrids were grown under control or heat stress conditions and exogenously applied with water and aqueous leaf (fresh and shade dried) and flower extracts using three different modes i.e., seed priming, medium supplementation and foliar spray. Data suggested that all the types and modes of moringa extract applications were substantially effectual in enhancing the osmolytes synthesis. Control plant of both the hybrids indicated a greater while heat stressed plants revealed a lesser accumulation of all the omsolytes. Among the osmolytes, the most conspicuous accumulation was recorded for shoot free proline and shoot GB, while TFA did not display much variation across the application modes. Among the extracts application modes, the most effectual ones were medium supplementation followed by foliar spray. Such accumulation patterns of the osmolytes were very important in aspect of the worth of both free proline and GB in improving the cell water balance and acting as osmoprotectants (Ashraf and Foolad, 2007; Hayat *et al*., 2012). Research has revealed that high temperature is an activator of free radicle production while free proline is a free radicle scavenger and thus helps in the stabilization of membrane structure. Likewise, GB, which is synthesized in the chloroplast and is more effectual at the site of synthesis. Thus better stabilization of chloroplastic membranes is important in the light harvesting and CO_2 fixation and thus better plant resistance in high temperature (Wahid, 2007; Gururani *et al*., 2015).

In this study great variations were observed in the type of extracts and their application mode, applied to determine the biosynthesis of different osmolytes. Among the extract types, MDLE was observed to be the most effectual, followed by fresh leaf extract of moringa plant, both under control and heat stress condition although heat stress crippled the synthesis of all the osmolytes in both the hybrids, nonetheless SB-11 (tolerant hybrid) behaved fairly better. This is assignable to the differences in the phytochemical composition of the extracts, where the MDLE seemed to contain such constituents which signaled the up-regulation of free proline and GB biosynthesis pathways in maize hybrids. However, other extract types apparently lacked such ability. Such a biosynthesis pattern of osmolytes was greatly important in the stress tolerance by the maize hybrids especially under heat stress. Moreover, among the modes of extract application the medium supplementation of extracts was substantially more effectual than foliar spray while seed priming was the least effectual. This may be attributed to the improved root growth with the application of extracts being directly exposed to it, leading also to the greater synthesis of free proline and GB in leukoplasts.

In conclusion, although all the aqueous extracts of moringa were effectual in improving the TFA, GB and free proline accumulation in both root and shoot, the MDLE was the most effectual extract type and medium supplementation was the most effectual mode for application of extract in improving the synthesis especially of free proline and GB. The differences in the synthesis of osmolytes may be attributable to the possible differences in the phytochemical constituents of extracts. Thus heat tolerance in maize and possibly other crops can be produced with the use of aqueous moringa extracts at optimized concentrations.

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