Chapter 2
Drought Tolerance in Some Field Crops: State of the Art Review

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Abstract Drought stress has a significant negative impact on plant growth, yield and quality of crop plants, particularly under current climate change. Therefore, improving drought tolerance in field crops is essential to increase sustains productivity particularly in arid and semi-arid regions in the Mediterranean basin. There are various selection criteria associated with drought tolerance could be exploited in selecting drought-tolerant genotypes. Combining morpho-physiological and biochemical traits can provide a more completed model of gene-to-phenotype relationships and genotype-by-environment interactions. Integration of recent advances breeding methods as quantitative trait loci, marker-assisted selection and genetic engineering technique with classic plant breeding helps significantly in developing drought-tolerant genotypes accurately and rapidly. This chapter also addresses to genetic diversity among genotypes, related traits to drought tolerance, genetic behavior, breeding efforts and biotechnology in rice, maize, barley and sunflower.

Keywords Drought · Field crops · Breeding methods · Biotechnology · Marker-assisted selection

2.1 Introduction

Global population is increasing while water resources for crop production are decreasing. Limited irrigation water is one of the major stresses that reducing crop production and quality in agricultural systems (Arshadi et al. 2018; Hasanuzzaman et al. 2018; Mansour et al. 2020). Moreover, the importance of drought has become more serious with increasing climatic change and global warming. The increase of air temperature and the decrease of rainfall caused heat stress and drought “in many areas, especially in arid and semi-arid regions” (http://www.tandfonline.com/doi/abs/10.1080/07900629208722539). Egypt suffers from severe water deficit in recent years, facing water shortage amounted about 7 billion cubic meters annually and

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H. Awaad et al. (eds.), *Mitigating Environmental Stresses for Agricultural Sustainability in Egypt*, Springer Water,
https://doi.org/10.1007/978-3-030-64323-2_2

this may increase in the near future due to the effect of Ethiopian Renaissance Dam (Osman et al. 2016). Drought tolerance is defined as the ability of the genotypes to produce acceptable yield under limited water supply better than the other genotypes (Desoky et al. 2020). Whereas, drought sensitivity is the reduction in yield of the genotypes under drought stress (Gavuzzi et al. 1997; Turner et al. 2001). Drought stress tolerance development is difficult due to the phenomenon of well-built interactions between genotypes and the environmental conditions. Therefore, based on yield loss under water stress conditions with compare to normal conditions, various drought measurements were determined that have been used for identification of drought-tolerant genotypes (Mitra 2001; Ali 2016). While other experiments yet have chosen a mid-point and think in selection under both favorable with combined stress conditions (Mardeh et al. 2006; Abdel-Ghany 2012). Marker-assisted selection approach serves the purpose of efficient transfer of the desirable gene into the elite of crop plants which infers sustainable saving in time compared to conventional backcross breeding. Marker-assisted selection such as RAPD, ISSR and real-time quantitative polymerase chain reaction (RT-qPCR) were used in breeding programs to assess the genetic relationships among genotypes and to improve biotic and abiotic tolerant traits (Nguyen et al. 1997; Premnath et al. 2016).

2.2 Rice

2.2.1 *Economic Importance*

Rice crop feeds approximately half of the world's population (Uga et al. 2013). The area allotted to rice crop in Egypt was 1.4 million feddan (feddan = 4200 m^2), and total production was 5.7 million tons of grains with an average yield of 4.0 tons fed^{-1} (Faostat 2018). The drought tolerance mechanism is complex, influenced by variants in plant phenology and controlled by numerous quantitative trait loci. Drought is a major constraint to the productivity of rice in worldwide. Drought stress is a major environmental limit to rice growth, production and yield in many regions of the 'world (Luo et al. 2009). Drought stress is a problem in nearly 45% of agricultural areas and the major worldwide restraint to productivity, making it an important and vital research space in scientific reports (Heinemann et al. 2015; Todaka et al. 2015).

Water deficit is an important constraint affecting rice physiological processes that are involved in its growth and development, as well as agricultural productivity. Given the importance of the rice crop economically, seems imperative to study the extent to which different genotypes are tolerated under water-stress conditions. The study of morphological, physiological and biochemical characteristics is important in this regard, in line with the objectives of breeding. Therefore, drought-resistant rice cultivars are an economically important target for crop production and food security.

2.2.2 Mean Performance and Genetic Diversity

Various field trials were conducted under normal and drought conditions to evaluate yield response of different genotypes to drought stress in addition to identify related traits to drought tolerance. Abd-Allah et al. (2010) assessed thirty-three local and exotic rice genotypes including eighteen Egyptian, six Italian and nine Chinese rice genotypes were evaluated under normal and drought conditions at the experimental farm of Rice Research and Training Center. The results revealed highly significant differences among the genotypes for all evaluated traits. Several promising genotypes were identified as drought tolerant under different growth stages, *i.e.* seedling, early and late vegetative, panicle initiation and heading stages. These genotypes displayed associated traits with drought tolerance as early maturity, intermediate tillering ability and plant height, root depth, root thickness, root volume, dry root: shoot ratio, plasticity in leaf rolling and unrolling. Additionally, these genotypes exhibited high water use efficiency and water application efficiency. These genotypes were Giza 178, Giza 182, GZ5121, GZ 6296-12-1-2-1-1, GZ 8310-7-3-2-1, GZ 8367-11-8-3-2, GZ 8372-5-3-2-1, GZ 8375-2-1-2-1, GZ 8450-19-6-5-3, GZ 8452-7-6-5-2, GZ 1368-S-4, Augusto and SIS R215.

El-khoby et al. (2014) crossed six rice genotypes with different water stress tolerance at the experimental farm of Rice Research and Training Center, Sakha, Kafr Elsheikh, Egypt. Three crosses viz; cross I [Tsuyuake (tolerant) × Sakha 103 (sensitive)], cross II [Zenith (tolerant) × Sakha 104 (moderate)] and cross III {BL1 (moderate) × Sakha 106 (sensitive)} were produced. Results showed that high variances between the six parents for all root characters, grain yield and its associated traits under water stress circumstances. Parent Tsuyuake produces the highest estimates for most traits, whereas, the lowest values were noted for Sakha 106 rice cultivar. The mean values of F_1 were higher than the highest parent for root volume, root/shoot ratio, in cross I, root length, root fresh weight, plant height and grain yield plant^{-1} in crosses I and II and number of panicles plant^{-1} in the three crosses.

To evaluate drought effect on rice growth and productivity, as well as the influence of silicon to enhance rice tolerance to drought stress. Ibrahim et al. (2018) performed an experiment in clay soil. They assessed five levels of soil moisture content (SMC) (70, 80, 90, 100, and 120% of the soil saturation point) and five rates of Silicon (Si) (0, 2.1, 4.2, 6.3, and 8.4 mg Si/10 plants). They detected significant reduction in plant height, rice straw, root yield, and grain yield by 32, 52, 36, and 27%, in the same order, with reducing SMC from 120 to 70%. On the other hand, Si significantly increased the aforementioned traits by 38, 97, 49, and 106%, respectively. The combination of SMC of 120% and 8.4 mg Si rate presented the maximum plant growth traits, while SMC of 70% and 0 mg Si rate recorded minimum plant growth traits. Furthermore, grain yield and aboveground biomass could be maintained under reducing water supply, if Si were supplied.

At the Rice Research and Training Center (RRTC), Sakha, Kafr Elsheikh, Egypt, during 2014 and 2015 growing seasons, Bassuony and Anis (2016) evaluated some root characters of five rice genotypes for drought and well-watered conditions. Genotypes were differed significantly by sowing conditions. Growth rate (g day^{-1}) varied considerably amongst the considered genotypes. Genotype Nerica 4 gave a much greater growth rate compared to other genotypes. Drought stress leads to a decreased total root length for all genotypes. Root volume decreased differentially with a range from 24.53 to 19.17 under the drought situation. Numbers of total roots differed significantly under the two sowing situations. Under well-watered conditions, the number of roots increased compared to drought situation. Under well-watered root, the dry weight was higher compared to drought. Also, root to shoot ratio decreased under drought stress.

Variation in root morphology, some physiological traits, yield and its components and genetic diversity among some Egyptian rice varieties and some upland rice accessions under normal and drought conditions were studied by Sedeek et al. (2010). Four upland rice accessions originated from Cote de Ivoire (IRAT112, IRAT170, WAB450-I-B-P-105-HB and Yun Len62) compared with three Egyptian rice varieties (Giza177, Sakha101 and GZ745613-6-5-3) were evaluated. Significant variation was observed in most of the investigated root traits such as root length, root volume, root thickness and root dry weight among the upland rice accessions and Egyptian rice varieties. Also, there are significant variation in some physiological traits which were related to fitness and productivity, including yield and its components under normal and drought conditions. This indicated that the upland rice accessions are tolerant to drought via its have good root system compared with the Egyptian rice varieties which were sensitive to drought. Most of the traits showed significant and highly significant correlation. Furthermore, among sixteen rice genotypes evaluated under drought and normal conditions, Gaballa (2018a) found that the effect of years was highly significant for leaf rolling and the number of tillers plant^{-1}. Genotype GZ 5310-20-3-3 had the desirable value of sterility percentage under normal conditions, while Giza 107 gave the best value under drought conditions. The heaviest mean value of 100-grain weight was recorded by E. Yasmine cv. Moreover, the highest grain yield plant^{-1} was produced by Giza 103, Giza 105 and Giza 107 under normal conditions, while Giza 107 and GZ 8372-5-3-2-1 gave the highest value under drought conditions. Also, Gaballa (2018b) tried four treatments, *i.e.* flash irrigation every 12 days, flash irrigation every 12 days + application of kinetin (1gL^{-1}), flash irrigation every 12 days + application of kinetin (2gL^{-1}) and normal irrigation. Registered highly significant differences among treatments, genotypes and their interaction in most traits. The most desirable mean values towards root length, root volume, number of roots plant^{-1}, days to heading, number of tillers plant^{-1} and number of panicles plant^{-1} resulted from Giza 179. Sakha 105 gave the best values of root: shoot ratio and chlorophyll content. The greatest values of leaf rolling, flag leaf area, plant height, harvest index and sterility percentage were recorded by GZ 8714-7-1-1-2. The superior values for plant height and panicle length were found by GZ 9781-3-2-2-6 as well as for highest root thickness, 100-grain weight and grain yield plant^{-1} by GZ 9792-13-1-1-2.

2.2.3 Related Traits to Drought Tolerance

The drought tolerant genotype is that has higher grain yield compared to the others under the same water deficit conditions. The improvement of breeding for drought tolerance in rice is slow due to the complication of the drought environment (Fukai and Cooper 1995). Several drought-tolerance mechanisms and characters were identified in rice *i.e.* drought escape through appropriate phenology, root traits, avoidance of specific dehydration and drought recovery. The physiological characters are considered very important for growth environmental conditions. The root system plays an essential role in water uptake and extraction from the deep soil layers. Therefore, root system traits *i.e.* deep root, high intensity root length in depth are valuable in this context. Ekanayake et al. (1985) presented that selection of root types must be successful in early segregating generations if the selection is proficient on such traits. Furthermore, relations between root traits and other agronomic traits as plant height, tiller number, and shoot weight were positive and significant. Root traits significantly associated with field drought tolerance as well as with leaf water potential that strengthening the role of root traits in maintaining high leaf water potential under drought stress. Abd-Allah et al. (2010) reported that certain evaluated agronomic traits as number of tillers plant^{-1}, number of panicles plant^{-1}, 100-grain weight and panicle weight indicated significant genotypic correlation with grain yield. Likewise, number of filled grains panicle^{-1} displayed highest direct contribution (0.63), and similarly it showed highest indirect contribution (0.867) followed by 100-grain weight (0.850) to grain yield. Path coefficient analysis demonstrated that number of panicles plant^{-1}, 100-grain weight, number of filled grains panicle^{-1}, panicle weight should be improved to increase grain yield under both normal and drought conditions. El-khoby et al. (2014) reported that grain yield plant^{-1} was highly significant and positively correlated with root length, root number plant^{-1}, root volume, panicle length, number of panicles plant^{-1}and 100-grain weight. On the other hand, the grain yield plant^{-1} was highly significant and negatively correlated with plant height, with similar results were reported by Muthuramu et al. (2010) and Hosseini et al. (2012). Under drought conditions, Gaballa (2018b) documented positive and highly significant correlation among rice grain yield plant^{-1} and other traits as root length, root volume, number of roots plant^{-1}, root thickness, root: shoot ratio, chlorophyll content, relative water content, flag leaf area, plant height, number of tillers plant^{-1}, panicle length, 100-grain weight, and number of panicles plant^{-1}. Furthermore, negative correlation has been detected with leaf rolling and sterility percentage.

Various stress-responsive genes, encoding antioxidant enzymes, oxido-reductases, kinases, and detoxification proteins, were prompted by drought and contributed to adjust the balance of reactive oxygen species ROS metabolism in the drought-tolerant cultivars (Degenkolbe et al. 2009; Yang et al. 2010). Besides, many genes were expressed under water stress lead to alternate physiological and phenotypic reactions amid genotypes (Lenka et al. 2011). Under water-limiting environments, the genotypes that reserve high leaf water potential usually present best growth. Nevertheless, it is not known whether genotypic variation in leaf water potential is uniquely caused by root traits or other reasons. Osmotic adjustment is promising, because it resists the effects of a rapid shortage of leaf water potential. There is a genotypic difference in expression of green leaf retention as physiological character related to prolonged droughts (Fukai and Cooper 1995). Additionally, Farid et al. (2016) reported that rice crosses Sakha 102 × Giza 178, Sakha 102 × A22, Giza 178 × WAB 54-125 and Sakha 105 × WAB 54-125 exhibited the highest performances of leaf chlorophyll content, proline content, number of panicles plant^{-1} and grain yield plant^{-1} under drought conditions, indicating to their importance in improving drought tolerance in rice.

Drought always causes oxidative injury to cells as a result of excessive generation of reactive oxygen species ROS in crop plants (Selote et al. 2004; Moumeni et al. 2011). The drought-tolerant rice genotypes showed lower H_2O_2 level and enhanced antioxidative enzyme activity compared to drought-sensitive ones (Rabello et al. 2008). Likewise, Zhang et al. (2012) proposed that the genotype Yangdao-6 seedlings showed drought tolerance associated with greater antioxidant enzyme activity and less ROS accumulation rather than Yangdao-2 genotype.

2.2.4 Genetic Behavior

Tolerance to water stress in rice is a complex trait (Fukai and Cooper 1995). Ekanayake et al. (1985) reported that the ability of rice genotypes to tolerate drought stress is related with root system traits. The inheritance of root traits of rice (*Oryza sativa* L.) was assessed using the parents, F_1, F_2 and F_3 populations of cross IR20 × MGL-2. Polygenic inheritance was detected for the root traits. The F1 plants had root systems described by thick, deep roots with a higher lateral and vertical distribution rather than the low parent (IR20), with positive and significant heterosis. The additive and dominant genetic effects were equally contributed to the expression of the traits. Progeny parent regression of F_3 and F_2 and the narrow sense heritability estimations in F_3 were high for root thickness (0.61 and 0.80), root dry weight (0.56 and 0.92) and root length density (0.44 and 0.77) as documented by Ekanayake et al. (1985). Consequently, selection of root types, based on individual plant performance, would be successful in early segregating generations. Besides, El-khoby et al. (2014) evaluated three crosses viz; cross I {Tsuyuake (tolerant) × Sakha 103 (sensitive)}, cross II {Zenith (tolerant) × Sakha 104 (moderate)} and cross III {BL1 (moderate) Sakha 106 (sensitive)}. They recorded highly significant positive heterosis and heterobeltiosis for some root traits and grain yield and its relevant traits especially in crosses I and II. The additive genetic component was higher than the dominance one for root volume and grain yield plant^{-1} in cross II, root fresh weight and days to 50% heading in crosses II and III, root:shoot ratio in crosses I and III, as well as, plant height and panicle length in the three crosses. Heritability in broad sense ranged from low to intermediate and high in the three crosses. However, it was low in the narrow sense. Furthermore, they recorded low genetic advance (3.99) for root number plant^{-1} to high (30.15) for root length in the cross III. Tsuyuake and Zenith might be useful genotypes in breeding program for water stress condition. Moreover, Bassuony and Anis (2016) assess some root traits of some rice genotypes for drought and well-watered conditions. The magnitude of heterosis manifested over mid-parent, and better parents exist. Highly significant estimates of heterosis as a deviation from mid-parent and better parent were revealed in all crosses for growth rate (g/day) and root length (cm) under normal and drought situations (Bassuony and Anis 2016). Zhang et al. (2012) identified numerous differentially expressed genes between seedlings of genotypes YD2 and YD6. For examples, *CuZnSOD*, *CAT-A*, *GSR-1*, *CYP71A1*, *CYP72A1*, *CYP72A5*, and *CYP86A1* were notably induced by drought in YD6, but mildly or not altered in YD2.

2.2.5 Breeding Efforts and Biotechnology

The genetic improvement of drought tolerance is addressed through the conventional approach by selecting for yield and its stability over locations and years. Du to of low heritability of yield under stress and inherent variation in the field, such selection programs are expensive and slow in getting progress. Nguyen et al. (1997) presented that the ability of root systems to provide for evapotranspiration demand from deep soil moisture and capacity for osmotic adjustment are considered main drought tolerance traits in rice. Selection for these traits still requires extensive investments facilities and is subjected to challenges of repeatability due to environmental inconsistency. Molecular markers associated with root traits and osmotic adjustment are being identified, that lead to marker-assisted selection. Furthermore, transgenic tolerant rice to water stress and osmotic stress have been documented. Moreover, various researches on genetic engineering of osmoprotectants, such as proline and glycine betaine, into the rice plant for drought tolerance improvement are in progress. Basi (2008) noticed expression of a putative tyrosine kinase gene (Pup1 candidate gene #43) under P and drought in NIL 14-4 but not in NIL 14-6 or Nipponbare. Tolerance specific gene #18 and gene #43 is absent from the Nipponbare genome but are present in drought-tolerant wild rice species from Asia and Africa, e.g. *O. longistaminata, O. barthii*, and *O. rufipogon*, proposing that these genes might be ancient and were lost in modern, stress-sensitive genotypes. Madabula et al. (2016) assessed the phenotypic response of 6 Brazilian rice varieties and 2 different crosses between them under drought conditions. Four genes related to auxin response and root modifications (*OsGNOM1 CRL4, OsIAA1, OsCAND1* and *OsRAA1*) were detected. Through real-time quantitative polymerase chain reaction (RT-q PCR), displayed that all genotypes lengthened its roots in response to drought, especially the derived 2 hybrids. The expression of these genes is modified in response to drought stress, and *OsRAA1* has a very special behavior, constituting a target for the future. Under Egyptian conditions, several drought-tolerant varieties have been developed through the National Rice Research Program characterized by a short growth period and water-saving *i.e.* Giza 179, Giza 182, Sakha 102, Sakha 103 and Sakha 107.

2.3 Maize

2.3.1 Economic Importance

Maize (*Zea mays* L.) is a major crop in the United States and worldwide. Drought is one of the most vital problems facing crop cultivation in most areas of the world. It is also the most important environmental limitations and pressures affecting the growth and productivity of maize plants. The production and stability of the crop are strongly affected by drought stress conditions. So, improved drought tolerance in maize is a top priority for corn breeding programs. In Egypt, the average productivity of maize was 8.3 tons ha^{-1} with a total production of about 8.6 million tons of grains (Faostat 2018). The performance of crops are a highly complex phenomenon under water stress condition and negative affected (Reynolds et al. 2006). Effects of water stress on maize depend on the growth stage of prevalence, duration of prevalence, and intensity of stress. Consequently, research on irrigation and water management has focused on crop productivity responses to water provide (Chen et al. 2010; Köksal 2011). It is interesting to mention that when drought stress starts to effect on the plant at the reproductive stage, the plant lessens the demand for carbon by decreasing the size of the sink. Therefore, reduction in leaf size, stem extension and root proliferation, the flower may drop pollen may die and ovule may abort (Blum 1996; Farooq et al. 2009). The yield reduction under drought stress was higher at the reproductive stage than at the vegetative and grain filling stages (Fatemi et al. 2006). Whereas, Khodarahmpour and Hamidi (2012) found that the production of maize grain yield reduced 60% due to stress condition at grain filling stages. Furthermore, Ali (2016) registered reduction % due to water stress valued (32.83%) for grain yield (ard./fed.), (15.60%) for plant height, (14.99%) for ear length, (13.99%) for ear leaf area, (12.17%) for 100-kernel weight, (10.63%) for number of kernels row^{-1}, (6.98%) for ear diameter, (6.64%) for leaf relative water content and (4.22%) for number of rows ear^{-1}.

2.3.2 Mean Performance and Genetic Diversity

Terminal drought stress is one of the most important environmental stress factors which can cause a significant reduction in maize production. Barutcular et al. (2016) conducted two field experiments with some maize hybrids in two cropping seasons, 2014 and 2015 under two moisture levels (normal irrigation and water deficit-stress) at the grain filling stage. Results revealed that, yield and major yield traits of hybrids harmfully affected due to terminal drought stress, and causing a reduction in yield with comparing normal irrigation situation. Water stress significantly affected on maize hybrids and there was high variation among hybrids, which could be befitting for screening the genotypes. Hybrids 71May69, Aaccel and Calgary were revealed less reduction in grain yield under terminal drought stress. Genotypes with high stress sensitivity index and tolerance index were expressed as highly sensitive to drought and only appropriate for irrigated conditions.

Mean values of the maize parental lines and their respective twenty crosses for earliness and grain weight plant^{-1} have been assessed under stress (D) and normal (N) conditions by Khaled et al. (2013). The results revealed that the mean performance of days to 50% tasseling for 9 parental lines varied from extreme earliness of line A3 (53.3 days in drought and 55.7 days in favorable environments) to lateness of line C12 (80.3 days in drought and 84 days in favorable environments). The cross (A3 × B3) had the best mean values for earliness (52.7 days and 54.3 days) under drought and normal stress conditions, respectively. As for grain weight plant^{-1}, the range of mean performances varied from 24.0 to 37.5 g for B8 and B5 lines, respectively under normal conditions. However, the range was narrower extending from 15.9 g for line B10 to 22.1 g for line B5 under drought stress. The crosses (C16 × C12) and (B10 × B3) had the highest grain yield under normal and drought stress conditions, respectively. The results of drought sensitivity index (DSI) displayed values of 0.38, 0.39, 0.74, and 0.99 for the parental lines B8, A3, C12 and C16, respectively, indicating relative drought resistance. At the same time, Maciej et al. (2013) revealed that the DSI values fluctuated from 0.381 to 0.650. While, B10 and C1 were the most sensitive maize parental lines. Regarding the F_1 crosses, 9 out of the 20 F1 crosses showed relative drought resistance.

During the three growing seasons 2011, 2012 and 2013 at Experimental Farm, Fac. of Agric., Zagazig University, Ali (2016) evaluated half diallel crosses among eight yellow maize inbred lines *i.e.* Z12 (P1), Z15 (P2), Z167 (P3), Z147 (P4), Z40 (P5), Z56 (P6), Z58 (P7) and Z103 (P8) under well-watered and water stress environments. Results presented in Table 2.1 showed that the following crosses had the most desirable sensitivity index to drought tolerance *i.e.* SC. 168, (P2 x P5), (P5 x P7) and (P5 x P8) for days to 50% silking; (P3 x P7), (P2x P6), (P4 x P6) and TWC.352 for anthesis to silking interval; (P7 x P8) for plant height; (P1 x P5), (P1 x P6) and (P1 x P8) for ear leaf area; (P2 x P3) and (P2 x P8) for leaf water content; (P2 x P5), (P1 x P7), (P1 x P6) and (P1 x P5) for ear diameter; (P5 x P6), (P2 x P7) and (P3 x P7) for ear length; (P3 x P6), (P3 x P7), TWC.352 and (P7 x P8) for number rows/ear; (P4 x P5), (P2 x P7) and (P7 x P8) for number of kernels/row; (P5 x P8), (P4 x P5) and TWC.352 for 100-kernels weight and (P2 x P7), (P3 x P7), (P5 x P6), (P1 x P6) and (P7 x P8) for grain yield.

In comparison among drought-sensitive and drought-tolerant maize genotypes in respect to morph physiological and biochemical characters components, Tůmová et al. (2018) exhibited that genotype CE704 had significantly higher values of number of visible leaves and plant height compared to maize genotype 2023 under drought conditions. While genotype 2023 subjected to drought attained strong symptoms of senescence for the older leaves, rather than genotype CE704, which developed normally even in stress situation. Drought significantly reduced the shoot dry mass and total leaf area in genotype 2023 nevertheless not in genotype CE704, resultant in genotypic variances under drought conditions. Also, the root biomass in stressed plants of genotype CE704 was a greater rather than genotype 2023. Drought stress was decreased leaf osmotic potential values in both genotypes compared to control.

Table 2.1 The mean performance of 28 F_1 maize crosses and two check varieties for drought sensitivity index (DSI) for all studied traits and drought tolerance index (DI) and stress tolerance index (STI) for grain yield only (Ali 2016)

Crosses	Days to 50% silking	Anthesis-silking interval(ASI)	Plant height (cm)	Ear leaf area (cm^2)	Leaf water content%	Ear diameter (cm)	Ear length (cm)	No. rows ear^{-1}	No. kernels /row	100—kernel weight(g)	Grain yield (ard. $fed.^{-1}$)		
											DSI	STI	DI
P1 X P2	2.11	2.47	0.75	0.42	0.86	0.59	0.72	2.05	1.74	1.46	1.06	0.58	0.60
P1 X P3	1.89	1.74	1.22	0.53	1.39	0.87	0.93	1.31	1.08	1.16	1.24	0.61	0.53
P1 X P4	1.54	0.81	0.98	0.87	1.09	0.91	1.23	0.89	1.56	0.86	1.08	0.77	0.67
P1 X P5	0.67	1.78	0.72	0.08	0.70	0.51	0.50	1.33	1.58	1.66	0.76	0.75	0.84
P1 X P6	1.28	−1.68	0.88	0.23	0.70	0.50	1.64	0.36	1.05	0.85	0.96	0.69	0.70
P1 X P7	0.68	4.74	0.85	1.37	0.86	0.47	1.89	0.49	1.05	1.96	1.03	0.70	0.67
P1 X P8	1.43	1.18	1.11	0.31	0.87	0.77	1.04	1.32	1.28	1.41	1.16	0.57	0.55
P2 X P3	0.86	4.31	1.01	0.71	0.52	−0.24	1.42	0.83	0.65	1.20	1.04	0.97	0.79
P2 X P4	1.02	1.35	1.09	1.33	1.74	−0.31	1.27	0.48	0.76	1.83	1.02	0.83	0.73
P2 X P5	0.38	−1.59	1.40	0.37	1.18	0.40	1.03	1.76	0.98	0.68	0.99	0.63	0.66
P2 X P6	1.72	0.14	1.16	0.94	0.95	0.85	0.57	0.55	0.77	1.10	1.04	0.46	0.54
P2 X P7	1.02	1.00	1.26	2.02	0.94	0.77	0.28	0.55	0.39	0.88	0.74	0.45	0.66
P2 X P8	1.24	0.82	0.82	1.76	0.52	0.68	1.17	0.93	0.83	0.96	0.93	0.61	0.68
P3 X P4	1.67	2.34	1.00	1.38	1.21	1.66	1.12	1.91	0.68	1.14	1.07	0.66	0.63
P3 X P5	0.64	0.95	1.20	0.92	1.05	1.11	1.56	−0.17	1.22	0.47	1.20	0.65	0.57
P3 X P6	0.57	3.47	0.93	0.67	1.35	2.43	1.36	0.11	1.46	0.72	1.01	0.84	0.75
P3 X P7	1.62	0.11	1.11	0.69	0.92	1.33	0.51	0.20	0.69	0.91	0.67	0.73	0.87
P3 X P8	1.24	1.63	0.96	0.89	0.64	1.14	1.02	1.25	0.68	0.41	0.95	0.57	0.64
P4 X P5	0.88	0.96	0.93	1.09	0.91	1.81	0.94	−0.17	0.17	0.31	0.91	0.65	0.71

(continued)

Table 2.1 (continued)

Crosses	Days to 50% silking	Anthesis-silking interval(ASI)	Plant height (cm)	Ear leaf area (cm^2)	Leaf water content%	Ear diameter (cm)	Ear length (cm)	No. rows ear^{-1}	No. kernels /row	100—kernel weight(g)	Grain yield (ard. fed.$^{-1}$)		
											DSI	STI	DI
P4 X P6	1.56	0.26	1.12	1.67	1.45	1.41	0.88	1.03	1.02	0.87	1.31	0.52	0.46
P4 X P7	0.57	3.32	0.77	2.20	0.77	0.63	1.21	1.49	0.79	1.23	1.00	0.81	0.74
P4 X P8	0.62	1.32	0.79	1.20	0.96	1.22	0.99	1.24	1.35	1.26	0.92	1.04	0.89
P5 X P6	0.86	−0.33	0.78	0.84	0.75	0.90	0.07	0.33	0.67	0.89	0.81	0.51	0.67
P5 X P7	0.32	1.12	1.09	0.69	0.65	1.89	0.73	1.35	0.51	0.71	0.84	0.56	0.69
P5 X P8	0.31	0.99	1.27	0.36	0.87	1.85	0.93	1.62	1.20	0.25	1.03	0.83	0.73
P6 X P7	0.94	0.78	1.17	0.32	0.62	1.14	0.99	1.04	0.98	0.11	1.03	0.47	0.55
P6 X P8	0.92	0.99	0.90	1.31	1.02	0.54	0.74	1.56	0.54	1.25	0.97	0.83	0.76
P7 X P8	1.14	1.11	0.65	1.08	1.45	0.79	0.53	0.31	0.39	0.91	0.92	0.47	0.60
Checks													
SC. 168	0.27	2.02	0.93	1.28	1.52	0.76	0.68	1.77	1.58	1.39	1.03	0.91	0.76
TWC. 352	0.55	0.28	0.98	1.03	1.33	1.38	1.05	0.16	1.37	0.40	0.97	0.71	0.71
LSD 0.05	0.88	2.53	0.37	1.09	0.66	1.31	0.86	ns	0.76	0.90	0.31	0.10	0.15
LSD 0.01	1.17	3.37	0.49	1.46	0.88	1.75	1.15	ns	1.01	1.20	0.41	0.13	0.20

Ardab maize = 140 kg grains, Feddan = 4200 m^2

Transpiration rate was reduced in genotype CE704 than in genotype 2023 after 14 days without watering. Also, both stomatal conductance and net photosynthetic rate were reduced by drought, and the changes in the mean values of these traits were less evident in genotype CE704 than in genotype 2023. This was revealed in the presence of significant differences among both maize genotypes in the stomatal conductance under drought conditions. The drought-stressed plants of genotype CE704 had more carotenoids and chlorophyll in the leaves compared to genotype 2023. Also, drought stress reduced the efficiency of the primary photosynthetic procedures. There were obvious variances between the drought-stressed plants and control, mainly for the factors describing the performance index PI_{ABS} and electron transport within the photosystem (PS) II reaction centre, such as $\psi_{E0,}$ φ_{P0} and φ_{E0}. These differences were more obvious in the genotype 2023.The drought stressed plants had higher values for cell membrane injury than in the control. This increase was higher in genotype 2023 than in genotype CE704. The genotype 2023 also exhibited slightly higher peroxidation of membrane lipids based on the malondialdehyde content (MDA) compared to genotype CE704. Regarding to proline content, genotype CE704 was also characterized by higher percent in the leaves compared to genotype 2023, and drought stress induced a further elevation of this osmoprotectant content in genotype CE704 (Tůmová et al. 2018).

2.3.3 *Related Traits to Drought Tolerance*

Maize is more sensitive than most other cereal crops to drought stresses at flowering stage, where yield losses can be severe through reductions or barrenness in kernels per ear (Bolanos and Edmeades 1996; Al-Naggar et al. 2000). Moussa and Abdel-Aziz (2008) tested maize genotypes Trihybrid 321 (drought sensitive) and Giza 2 (drought-tolerant) for water stress condition produced by irrigating the pots with polyethylene glycol (PEG) solutions of 0.0, -5, -10 and -20 bars. The two genotypes responded differently under water stress and control conditions. The tolerant genotype Giza 2 displayed lower accumulation of malondialdehyde (MDA) and H_2O_2 content related to increasing activities of catalase, peroxidase compounds, and superoxide dismutase, under water stress situations. The superoxide dismutase activity as antioxidant increased constantly with increasing drought in both the genotypes, but the percent of antioxidant was higher in Giza-2 drought-tolerant. The lower membrane injury and higher water retention capacity have been detected in Giza-2. Further, Trihybrid 321 displayed resistance to water stress via the above adjustments. Water stress was resulted by the accumulation of free proline and gylycinebetain in both cultivars. The level of enhancement in both omsolytes was lower in Trihybrid 321 than Giza 2. Therefore, the accumulation of gylycinebetaine and free proline in the maize leaves genotypes can be used as the possible indicator for water stress tolerance. Giza 2 had higher relative water content under both water stress and non-stress conditions. Thus, the high relative water content is helping the tolerant maize genotype to achieve physio-biochemical processes more efficiently under drought environments than sensitive genotype. While, the contents of endogenous brassinosteroids (BRs) were considered in two genotypes differed in their water stress sensitivity. The attendance of 28-norbrassinolide in rather high quantities (1–2 pg. mg^{-1} fresh mass) is reported for the first time in the leaves of monocot plants. The drought-resistant genotype was considered by a significantly higher content of total endogenous BRs (28-norbrassinolide and particularly typhasterol) compared with the drought-sensitive genotype which exhibited higher levels of 28-norcastasterone. The differences observed between CE704 and 2023 genotypes in content of total endogenous BRs are probably related with their different levels of drought sensitivity, which was confirmed at different levels of plant biochemistry, physiology and morphology (Tůmová et al. 2018).

Tolerant maize genotypes were characterized by having shorter anthesis to the silking interval (ASI) (Bolaños and Edmeades 1993; Ali 2016), greater number of kernels ear^{-1} and more ears $plant^{-1}$ (Ribaut et al. 1997). Barutcular et al. (2016) reported a positive relationship between stress indices, grain yield, geometric mean productivity (GMP), drought resistance index (DRI), harmonic mean (HM), stress tolerance index (STI), mean production (MP), and Yield index (YI). Hereby could be used as the best selection indices for identifying the tolerant maize genotypes under terminal drought stress.

Under drought situations, Ziyomo and Bernardo (2013), recorded strongest genetic correlation between anthesis to silking interval (ASI) and grain yield (−0.77). Grain yield also under drought stress was strongly associated with leaf chlorophyll content, plant height, and leaf senescence. The genetic correlation was 0.61 between grain yield in the drought and control experiments, indicating that inbred lines with superior testcross performance in control condition also tend to perform well under drought environment.

Ahmed (2013) added that correlation coefficients between the studied variables showed that only the ear height and number of kernels row^{-1} were negatively correlated with grain yield and the highest correlations were observed between grain yield and grain weight under drought condition. While under control conditions, number of kernels/m^2 was highly associated with grain yield, thus, the hybrids with larger number of kernels should be selected to increase grain yield under irrigated condition. Whereas, number of kernels row^{-1} and grain weight ear^{-1} could be used as an important traits for improvement grain yield productivity under drought stress at the grain growth stage.

To identify secondary characters for selection of high grain yield under high plant density joint with water stress at flowering, Al-Naggar et al. (2016) chose six different maize inbred lines in tolerance to high density and drought stress at flowering (three lines tolerant *i.e.* Sk-5, L-20 and L-53, and three lines sensitive *i.e.* Sd-7, L-18 and L-28) for diallel crosses. During two seasons, parents and hybrids were evaluated under two different environments; well-watered and low density of 47,600 plants ha-1 and drought stress with high density of 95,200 plants ha-1. They recorded strong favorable and significant genetic correlations between stress tolerance index or grain yield $plant^{-1}$ with all yield components for inbred lines and crosses and days to anthesis, ear height, plant height, leaf angle and barren stalks for crosses. Therefore, low days to anthesis, leaf angle, ear height, and high rows ear^{-1}, 100-kernel weight, kernels row^{-1}, kernels plant^{-1}, ears plant^{-1}, might be considered secondary characters to drought and high-density tolerance. The optimum selection environment for grain yield plant^{-1} is the drought and high-density tolerance environment for crosses and well-watered and low density environment for inbred lines. Moreover, Ali (2016) recorded positive and significant genotypic and phenotypic correlations between grain yield and each of leaf relative water content (0.488** and 0.307**), ear leaf area (0.443** and 0.355**), ear length (0.783** and 0.647**), ear diameter (0.691** and 0.546**), number of rows ear^{-1} (0.291* and 0.237), number of kernels row^{-1} (0.486** and 0.451**), 100-kernels weight (0.659** and 0.543**) and drought sensitivity index (0.484** and 0.388**, respectively), but had negative correlations with days to 50% silking (−0.034 and 0.004) and anthesis to silking interval (−0.572** and −0.491**, respectively) (Table 2.2). Path coefficient analysis showed that ear length exhibited the largest direct effect on grain yield (0.340) followed by drought sensitivity index (0.251), leaf relative water content (0.231), ear leaf area (0.182), number of kernels row^{-1} (0.171), ear diameter (0.135) and number of rows ear^{-1} (0.104) as shown in Table 2.3.

2.3.4 Genetic Behavior

Additive gene action and partial dominance were revealed for harvest index and plant height under normal and stress conditions, however the over-dominance was recorded for 100-grain weight and kernels ear row^{-1} (Hussain 2009). Furthermore, Khaled et al. (2016) showed that parents versus crosses, as an indication of average heterosis over crosses were highly significant under the two environments for the studied traits. The two main effects of "females" and "males" were highly significant under drought and normal conditions for all traits, indicating the prevailing of additive gene action. Mean square due to the "males × females" interaction was also highly significant in both conditions, reflecting the importance of dominance variance in the genetics of these characters. Estimates of heterosis showed that flowering of 9 and 10 out of 20 crosses were significant flowered than their mid-parents with negative heterosis values varied from (−4.07% to −20.56%) and (−2.94% to −19.29%) under normal and drought environments, respectively. As for grain weight, estimates of heterosis were positive and highly significant for all crosses under both conditions. Heterotic values ranged from 28.45% to 208.36% for crosses (C16 × B5) and (C16 × C12), respectively under normal conditions. Whereas, the heterotic estimates were increased and varied from 78.45% to 286.03% for crosses (C15 × B3) and (B10 × B3), respectively under drought conditions. Commonly, the superiority of some crosses over their mid parents reflects the important role of non-additive genetic variance in the inheritance of these traits.

General combining ability effects (GCA) was estimated by Ali (2016) as shown in Table 2.4, as combined over two environments normal and drought stress. Positive GCA effects were desirable for all studied traits, except for the silking date, anthesis

Table 2.2 Genotypic (rg) and phenotypic (rph) correlation coefficients as calculated from the combined analysis of variance of various metric traits in yellow maize genotypes across two environments (Ali 2016)

		Anthesis-silking interval (ASI)	Plant height (cm)	Ear leaf area (cm^2)	Leaf water content %	Ear diameter (cm)	Ear length (cm)	No. rows ear^{-1}	No. kernels row^{-1}	100 kernel weight (g)	DSI for grain yield	Grain yield (ard. $fed.^{-1}$)
Days to 50% silking	rg	0.342^{**}	−0.257	0.228	0.325^{*}	−0.177	−0.158	-0.337^{**}	0.083	−0.102	−0.009	−0.034
	rph	0.236	−0.120	0.089	0.222	−0.126	−0.071	−0.147	0.052	−0.062	−0.011	0.004
ASI	rg	1.000	0.023	−0.159	−0.122	-0.330^{*}	−0.226	−0.213	-0.350^{**}	-0.383^{**}	0.165	-0.572^{**}
	rph	1.000	0.025	−0.139	−0.053	-0.304^{*}	−0.212	−0.193	-0.333^{*}	-0.333^{*}	0.079	-0.491^{**}
Plant height	rg		1.000	0.555^{**}	-0.276^{*}	0.425^{**}	0.628^{**}	−0.209	0.078	0.398^{**}	0.621^{**}	0.166
	rph		1.000	0.482^{**}	−0.137	0.356^{**}	0.510^{**}	−0.144	0.069	0.335^{**}	0.207	0.157
Ear leaf area	rg			1.000	−0.125	0.212	0.546^{**}	−0.216	0.482^{**}	0.634^{**}	0.031	0.443^{**}
	rph			1.000	−0.099	0.149	0.433^{**}	−0.160	0.416^{**}	0.542^{**}	−0.011	0.355^{**}
Leaf water content %	rg				1.000	0.343^{**}	0.050	0.176	−0.208	−0.026	0.558^{**}	0.488^{**}
	rph				1.000	0.097	0.045	0.107	−0.125	0.008	0.119	0.307^{*}
Ear diameter	rg					1.000	0.719^{**}	0.519^{**}	0.197	0.517^{**}	0.809^{**}	0.691^{**}
	rph					1.000	0.536^{**}	0.431^{**}	0.127	0.374^{**}	0.324^{*}	0.546^{**}
Ear length	rg						1.000	−0.011	0.453^{**}	0.729^{**}	0.829^{**}	0.783^{**}
	rph						1.000	−0.008	0.412^{**}	0.617^{**}	0.345^{**}	0.647^{**}
No. Rows/ear	rg							1.000	−0.035	−0.023	0.246	0.291^{*}
	rph							1.000	−0.069	0.032	0.079	0.237
No. kernels/ Row	rg								1.000	0.428^{**}	−0.252	0.486^{**}
	rph								1.000	0.376^{**}	−0.042	0.451^{**}

(continued)

Table 2.2 (continued)

		Anthesis-silking interval (ASI)	Plant height (cm)	Ear leaf area (cm^2)	Leaf water content %	Ear diameter (cm)	Ear length (cm)	No. rows ear^{-1}	No. kernels row^{-1}	100 kernel weight (g)	DSI for grain yield	Grain yield (ard. $fed.^{-1}$)
100 kernel	rg									1.000	0.439^{**}	0.659^{**}
	rph									1.000	0.201	0.543^{**}
DSI for grain yield	rg										1.000	0.484^{**}
	rph										1.000	0.388^{**}

*, ** Significant at $P = 0.05$ and $P = 0.01$, respectively Ardab maize = 140 kg grains, Feddan = 4200 m^2

Table 2.3 Direct (Diagonal) and indirect effects of some agronomic traits on grain yield of yellow maize genotypes across two environments relative to phenotypic correlation (rph) (Ali 2016)

Characters	Days to 50% silking	Anthesis-silking interval (ASI)	Plant height (cm)	Ear leaf area (cm^2)	Leaf water content %	Ear diameter (cm)	Ear length (cm)	No. rows ear^{-1}	No. kernels row^{-1}	100 kernel weight (g)	DSI for grain yield	Correlation with grain yield
Days to 50% silking	0.034	−0.065	0.020	0.016	0.051	−0.017	−0.024	−0.015	0.009	−0.002	−0.003	0.004
Anthesis-silking interval (ASI)	0.008	−0.277	−0.004	−0.025	−0.012	−0.041	−0.072	−0.020	−0.057	−0.010	0.020	−0.491
Plant height	−0.004	−0.007	−0.169	0.088	−0.032	0.048	0.173	−0.015	0.012	0.010	0.052	0.157
Ear leaf area	0.003	0.039	−0.081	0.182	−0.023	0.020	0.147	−0.017	0.071	0.016	−0.003	0.355
Leaf water content %	0.007	0.015	0.023	−0.018	0.231	0.013	0.015	0.011	−0.021	0.000	0.030	0.307
Ear diameter	−0.004	0.084	−0.060	0.027	0.022	0.135	0.182	0.045	0.022	0.011	0.082	0.546
Ear length	−0.002	0.059	−0.086	0.079	0.010	0.072	0.340	−0.001	0.070	0.019	0.087	0.647
No. Rows/ear	−0.005	0.054	0.024	−0.029	0.025	0.058	−0.003	0.104	−0.012	0.001	0.020	0.237
No. kernels/row	0.002	0.092	−0.012	0.076	−0.029	0.017	0.140	−0.007	0.171	0.011	−0.011	0.451
100 kernel	−0.002	0.092	−0.056	0.099	0.002	0.050	0.210	0.003	0.064	0.030	0.051	0.543
DSI for grain yield	0.000	−0.022	−0.035	−0.002	0.028	0.044	0.117	0.008	−0.007	0.006	0.251	0.388
Residual =		0.495										

Table 2.4 General combining ability (GCA) effects for grain yield and other agronomic traits combined over two environments (Ali 2016)

Inbred lines	Days to 50% silking	Anthesis-silking interval (ASI)	Plant height (cm)	Ear leaf area (cm^2)	Leaf water content%	Ear diameter (cm)	Ear length (cm)	No. rows ear^{-1}	No. kernels row^{-1}	100 kernel weight(g)	Grain yield (ard. $fed.^{-1}$)
P1 (Z12)	−0.29	-0.23^{*}	−3.50	-25.41^{**}	0.17	0.16^{*}	-0.48^{*}	1.36^{**}	−0.09	−0.61	0.15
P2 (Z15)	0.90^{*}	-0.44^{**}	−0.45	-17.24^{**}	0.05	0.08	−0.12	−0.48	−0.41	−0.15	−0.45
P3 (Z167)	−1.02	0.12	3.12	−2.28	0.67	0.21^{*}	0.60^{*}	2.16^{**}	-1.06^{*}	−0.12	0.89^{*}
P4 (Z147)	0.26	−0.11	23.63^{*}	47.82^{**}	−0.20	0.17^{*}	1.87^{**}	−0.22	1.15^{*}	1.98^{**}	1.43^{*}
P5 (Z40)	1.11^{*}	0.42^{**}	−2.58	34.45^{*}	0.19	−0.07	-0.80^{*}	-0.88^{*}	0.39	−0.17	−0.27
P6 (Z56)	0.20	0.40^{**}	−1.98	−7.60	0.06	-0.26^{*}	−0.28	-1.66^{**}	-1.99^{**}	−0.64	-0.92^{*}
P7 (Z58)	-1.57^{*}	-0.25^{*}	1.49	−11.73	-1.22^{*}	-0.16^{*}	-0.58^{*}	-0.69^{*}	0.11	−0.48	-1.35^{*}
P8 (Z103)	0.42	0.09	-19.73^{**}	-18.01^{*}	0.28	-0.12^{*}	−0.22	0.41	1.91^{*}	0.20	0.53
LSD $_{0.05}$ (gi)	0.85	0.15	3.76	15.05	0.72	0.11	0.39	0.50	0.74	0.66	0.61
LSD $_{0.01}$ (gi)	2.23	0.39	9.85	39.44	1.88	0.30	1.02	1.31	1.94	1.72	1.59
LSD $_{0.05}$ (gi-gi)	1.29	0.23	5.69	22.76	1.09	0.17	0.59	0.75	1.12	0.99	0.92
LSD $_{0.01}$ (gi-gi)	3.38	0.59	14.90	59.63	2.85	0.45	1.55	1.98	2.94	2.60	2.40

*, ** Significant at $P = 0.05$ and $P = 0.01$, respectively Ardab maize = 140 kg grains, Feddan = 4200 m^2

to the silking interval and plant height which exhibited negative values indicate a tendency towards earliness and shortness. Therefore, short plant height might be more resistant to stalk breakage, lodging and increasing plant density. The results indicate that, the parental line P7 (Z58) possessed negative and significant GCA effects for days to 50% silking, P1 (Z12), P2 (Z15) and P7 (Z103) for anthesis to silking interval, in the desired direction of earliness as well as P8 (Z103) for short plants. With respect to the ear leaf area, P4 (Z147) and P5 (Z40) had positive and significant GCA effects. None of the parents recorded positive and significant GCA effects for leaf water content. Positive and significant GCA effects were observed in P1 (0.16), P3 (0.21) and P4 (0.17) for ear diameter; P3 (0.6) and P4 (1.87) for ear length; P1 (1.36) and P3 (2.16) for number of rows ear^{-1}; P4 (1.15) and P8 (1.91) for number kernels row^{-1}; P4 (1.98) for 100-kernels as well as P3 (0.89) and P4 (1.43) toward higher-yielding ability. This result indicated that the two inbred lines P3 (Z167) and P4 (Z147) could be considered as good combiners for improving hybrids with yielding ability as well as inbred lines P7 (Z58) and P8 (Z103) for earliness and short plants, respectively.

Added that the ratio of GCA/SCA variances was more than unity for days to 50% silking, plant height and number of rows ear^{-1}, indicating the major role of additive gene effects in controlling the genetic mechanism of these characters over water environments. In contrast, the ratio of variance GCA to variance SCA was blown one for anthesis to the silking interval, ear leaf area, leaf relative water content, ear diameter, ear length, number of rows ear^{-1}, number of kernels row^{-1}, 100-kernel weight and grain yield. This emphasized that non-additive gene action was the prevailed type in controlling these traits. Narrow sense heritability estimates were high (> 50%) for days to 50% silking, plant height, ear diameter and number of rows ear^{-1}, moderate for ear leaf area (41.68%) and ear length (45.55%), and low (< 30%) for anthesis to silking interval, leaf relative water content, number of kernels row^{-1}, 100-kernel weight and grain yield over two environments. Moreover, Al-Naggar et al. (2016) recorded high narrow sense heritability (h^2n) for days to anthesis, leaf angle, ear height, rows ear^{-1}, kernels plant^{-1}, kernels row^{-1}, 100-kernel weight and ears plant^{-1} under both drought stress and high density of 95,200 plants ha^{-1} and well-watered and low density of 47,600 plants ha^{-1} environments.

2.3.5 Breeding Efforts and Biotechnology

The genetic diversity has been considered more efficiently linking polymorphism from the DNA, morphological and biochemical labels. Genetic variability in maize genotypes has been measured by morphological characters (Louette and Smale 2000; Beyene et al. 2005). DNA polymorphism analyses are great tools for studying and describing germplasm resources (Powell et al. 1996). Random amplified polymorphic DNA (RAPD) markers have been used in describing genetic diversity among maize genotypes (Moeller and Schaal 1999; Beyene et al. 2005). The level of association between DNA marker-based genetic similarity and agronomic description might differ between different crop species. In maize, a close correlation was established (Messmer et al. 1993). Hence, it is necessary to determine within each species whether agronomic characterization and DNA marker-based genetic similarity gave similar information about the genetic divergence among existing germplasm. In this respect, Okumus (2007) used 160 primers were to screening maize accessions and 14 primers were found to be valuable for RAPD assay and were exploited to amplify genomic DNA of 17 maize accessions. A total of 62 fragments were produced by seven primers with an average of 8.86 fragments per primer. The bands created in the RAPD experiments were strong and weak. The range of number amplification bands by each primer varied from 5 (OPW-08) to 13 (OPAT-08). The range of these fragments is in 99 (OPW-08) to 943 bp (OPAT08). Two maize inbred lines Sd-63 and Gm-18 were selected as drought-tolerant and drought sensitive, respectively to obtain the F_1 generation and then selfed to produce the F_2 generation. The two inbred lines and their F_1 and bulks of the two extreme F_2 plant groups (the most sensitive F_2 group and the most tolerant F_2 group) were verified against six RAPD primers. This analysis revealed that, four RAPD primers (A01, A05, A06 and B08) out of the six developed molecular markers for drought tolerance in maize. These RAPD markers can be expressed as reliable molecular markers related to drought tolerance in maize (Ahmed 2013). Bawa et al. (2015) revealed that the parent populations TAIS03, GUMA03-OB, IDW-C3-SYN-F2 and KOBN03-OB, and F1 hybrid populations IWD x GUMA03, IWD x TAIS03, IWD x KOBN03, TAIS03 x IWD, DT x TAIS03, DT x SISF03, DT x KOBN03, TAIS03 x DT, TAIS03 x SISF03, SISF03 x GUMA03, SISF03 x KOBN03, SISF03 x TAIS03, GUMA03 x DT,GUMA03 x KOBN03 and GUMA03 x IWD displayed bands across 9 drought-linked SSR markers. Thus, they contain the quantitative traits responsible for drought tolerance. Moreover, Li et al. (2016) identified the QTLs that control drought tolerance-related traits under well-watered conditions and water-stressed conditions using joint linkage analysis of the CN-NAM population. They identified 8–23 QTLs for the seven drought-related traits under well-watered, explained 23.7–66.3% of the total phenotypic variation. However, they recognized 8–20 QTLs under water-stressed conditions, explained 20.2–55.4% of the total phenotypic variation (Ahmed 2013). The genome-wide association analysis recognized 365 single nucleotide polymorphisms (SNPs) related to drought-related traits, and these SNPs were found in 354 candidate genes. Fifty-two of these genes exhibited significant variation expression in the inbred line B73 under the well-watered and water-stressed conditions.

In Egypt, Agriculture Research Center has succeeded in producing high yielding hybrid cultivars of white maize *i.e.* single crosses 10, 128, 129, 130, 131 and 132, and three-way crosses 310, 314, 321, 324 and 329, and the yellow maize crosses, such as single crosses 162, 166, 167, 168, 173, 176, 178 and 180 and three-way crosses of 352, 353 and 368 (Anonymous 2019).

2.4 Barley

2.4.1 Economic Importance

Barley is one of the oldest cultivated crops in the world with a high adaptive capacity. It displays high tolerance to adverse environmental conditions compared to other cereal crops (Gürel et al. 2016; Yang et al. 2017). Consequently, it is commonly grown in marginal areas and lands suffering water shortage and salinity. It is one of the most important crops with uses ranging from food and feed production representing the fourth most abundant cereal acreage and production after wheat, maize and rice (Faostat 2018). It is principally used for animal and poultry feeding, in addition to malt and some uses in the pharmaceutical industry. It contains 3–7% β-glucan, which is a very important dietary fiber that has health benefits (Oscarsson et al. 1996). For that reason, recently there is an increasing interest for human consumption due to its nutritional and healthy values especially hull-less barley as an ideal type for achieving this goal (Biel and Jacyno 2013).

Global population is increasing while water resources for crop production are decreasing. Limited irrigation water is one of the major stresses that reduces crop production and quality in agricultural systems (Arshadi et al. 2018; Hasanuzzaman et al. 2018). Moreover, the importance of drought has become more serious with increasing climatic change and global warming. The increase of air temperature and the decrease of rainfall caused heat stress and drought in many areas, especially in arid and semi-arid regions. Egypt suffers from severe water deficit in recent years, facing water shortage amounted about 7 billion cubic meters annually, and this may increase in the near future due to the effect of Ethiopian Renaissance Dam (Osman et al. 2016).

The drought has different impacts over barley growing stages. During the reproductive development stage, drought is a key factor affecting number of spikes/m^2. During spikelet initiation, drought leads to reduce grain set and grain number spikelet^{-1}, while during grain filling period it leads to reduce individual grain weight. Moreover, drought at the beginning of grain filling has negative effects on grain weight and grain yield more than during the late grain filling period (Samarah et al. 2009).

Drought tolerance is defined as the ability of the genotypes to produce acceptable yield under limited water supply better than the other genotypes. Whereas, drought sensitivity is the reduction in yield of the genotypes under drought stress (Turner et al. 2001; Desoky et al. 2020).

2.4.2 Mean Performance and Genetic Diversity

Barley germplasm provides a very fruitful source of genes and rich sources of genetic variation for improving drought tolerance. The genotypes exhibit different ability to produce acceptable yield under water deficit conditions. Accordingly, it is essential to screen the genetic potentiality of these genotypes under different water regimes. Additionally, evaluation the performance of barley genotypes under stress as well as favorable conditions is important at the beginning of breeding programs to identify suitable genotypes for environments, which helps in improving crop productivity (Mansour et al. 2017).

Various trials were performed under normal and water deficit conditions to display the genotypic variation and identify drought-tolerant and sensitive genotypes. In this context, Mansour et al. (2017) evaluated seventeen barley genotypes under different irrigation levels using drip irrigation system under sandy soil conditions. The treatments included four irrigation levels (severely-low 1200 m^3 ha^{-1}, low 2400 m^3 ha^{-1}, medium 3600 m^3 ha^{-1}, and high 4800 m^3 ha^{-1}). Plants exposed to water stress showed a significant decrement in plant height and yield attributes in comparison with well-watered plants. The drought-tolerant genotypes managed to produce more yield with higher water use efficiency (WUE) compared to drought-sensitive genotypes. Furthermore, the genotypes were classified into three groups using clustering procedure based on grain yield and four drought tolerance indices (mean productivity (MP), geometric mean productivity (GMP), stress tolerance index (STI) and yield index (YI)) (Fig. 2.1). The first group (a) included six genotypes which presented high tolerance indicesm and they considered as drought-tolerant genotypes (Giza-123, Giza-124, Giza-126, Giza-133, Giza-127 and Giza-128). The second group (b) contained

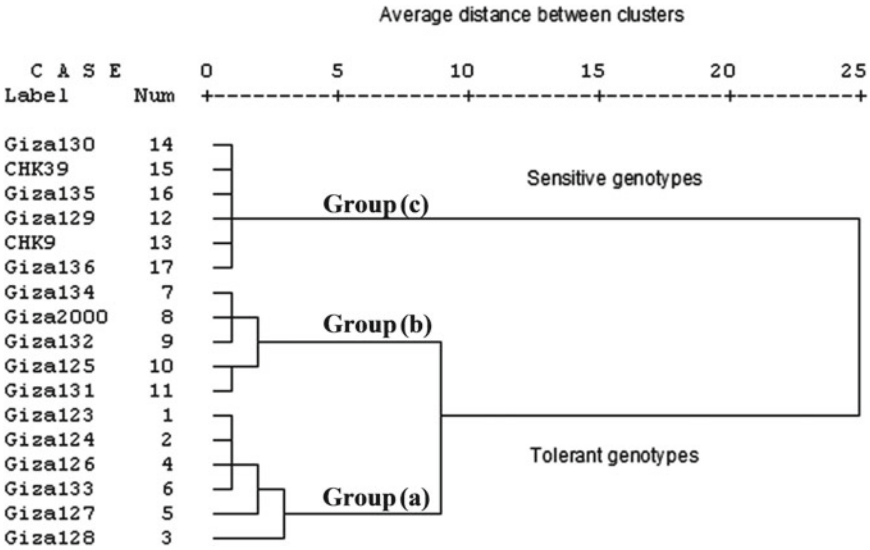

Fig. 2.1 Dendrogram of the phenotypic distances among seventeen barley genotypes under water stress and non-water stress conditions based on grain yield and the drought-tolerant indices, with cutting dendrogram obtained from Ward method in distance five. Group (a) represents drought tolerant genotypes, group (b) represents moderate drought-tolerant genotypes, and group (c) represents drought-sensitive genotypes (Mansour et al. 2017)

five genotypes which intermediated group in the drought-tolerance indices and were considered as moderate drought-tolerant genotypes (Giza-134, Giza-2000, Giza-132, Giza-125 and Giza-131). And finally the third group (c) presented six genotypes which displayed lowest tolerance indices and were considered as drought-sensitive genotypes (Giza-130, CHK-39, Giza-135, Giza-129, CHK-9, and Giza-136).

Furthermore, the results showed that drought-tolerant genotypes (group a) produced much greater grain yield and aboveground biomass with less irrigation water amounts compared with drought-sensitive genotypes (group c). Greater yield in drought-tolerant genotypes could be explained by greater yield attributes for those genotypes under water stress conditions (Fig. 2.2).

Additionally, the performance of fifteen barley genotypes under normal and under limiting water was studied by EL-Shawy et al. (2017). The evaluated genotypes included twelve genotypes from ICARDA, Egyptian landrace and two local varieties (Rihane-3 and Giza-126). Under limited water, the genotypes were irrigated only at sowing using approximately 1200 m^3/ha, (water stressed environment), while under normal conditions, the genotypes were irrigated at sowing (1200 m^3 ha^{-1}), followed by 1800 and 1850 m^3 ha^{-1}, 45 and 75 days after sowing, respectively. Result revealed sufficient genetic variability among the genotypes and importance of selection under stress conditions. The mean squares of irrigation regimes explained most of the variations for all the traits in both growing seasons, indicating the importance of evaluating genotypes under different irrigation regimes. Water deficit highly affected yield and its components in both growing seasons. Moreover, it was observed that among the studied genotypes, Line-2 had early heading and maturity which indicates that this genotype could be used as a source of earliness in breeding program. Besides, the genotypes; Line-7 and Line-11 exhibited the highest yield potential under water deficit in both seasons. This indicates that these genotypes could be considered drought-tolerant genotypes and could be exploited in the barley breeding programs for developing high yielding and drought-tolerant varieties.

Likewise, the response of fifteen barley genotypes under normal and water stress conditions was assessed by Mariey and Khedr (2017). The normal condition was irrigated twice after sowing; *i.e.* 45 days after sowing (at the tillering stage) and 75 days after sowing (at booting stage). While the water stress condition was given just sowing irrigation. They found that Giza-2000, Giza-126 and Giza-131 had the highest performance for grain yield and its components under normal and water stress conditions. Therefore these genotypes could be considered drought-tolerant and they could be recommended for using in breeding program for realizing high-yielding genotypes under normal and water stress conditions.

Moreover, El-Hashash and Agwa (2018) tested sixteen barley genotypes including fourteen exotic genotypes from ICARDA and two checks cultivars (Giza-123 and Giza-2000) under different stress conditions. The used genotypes were evaluated under severe drought stress, moderate stress and non-stressed conditions. The severe stress experiment was irrigated only once at sowing. While, the non-stress experiment was irrigated three timesm *i.e.*, at sowing, after 30 days from sowing (at the tillering stage) and after 75 days from sowing (at booting stage). On the other hand, the moderate stress experiment was irrigated twice at sowing and after 30 days from

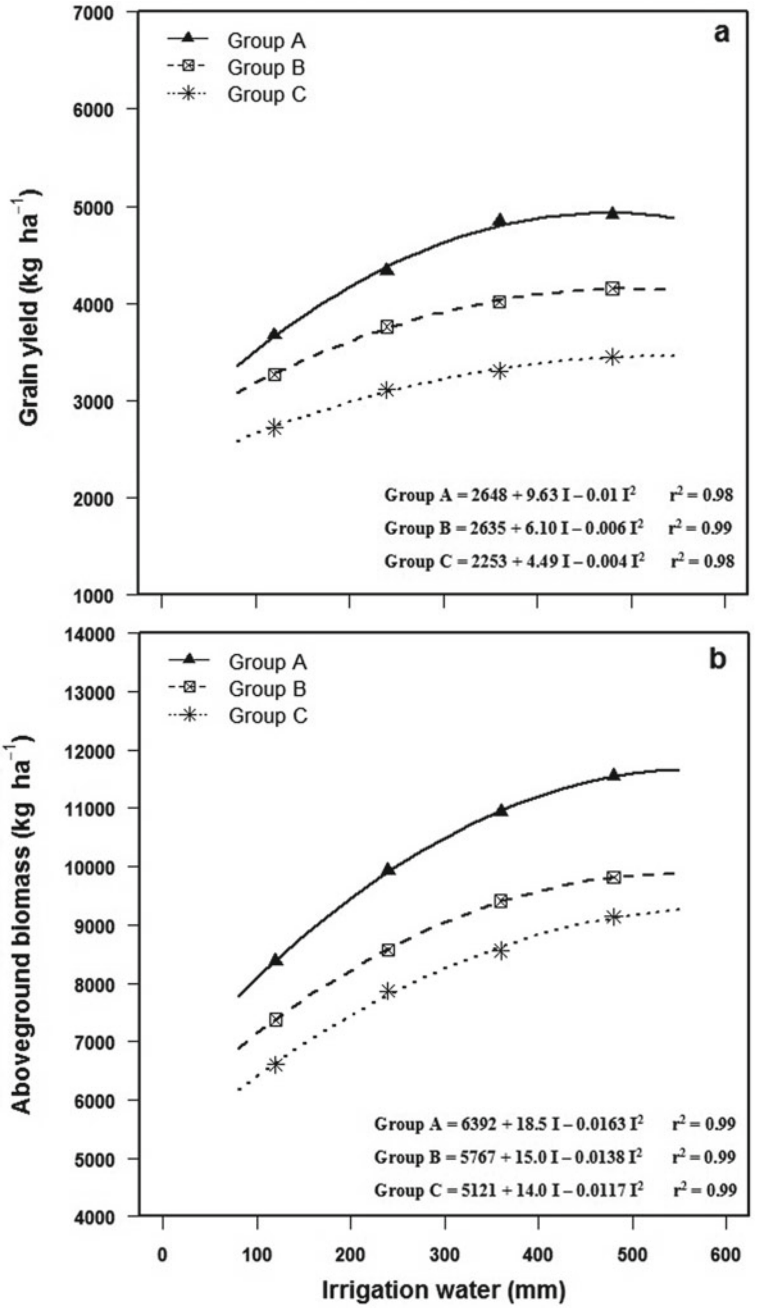

Fig. 2.2 Grain yield (a) and aboveground biomass (b) of the three groups of barley genotypes in response to irrigation water (Mansour et al. 2017)

sowing. The results revealed that all studied traits exhibited high performance under non-stress, followed by mild stress and then severe stress for most investigated genotypes. Most evaluated genotypes were better than the checks cultivars under the three irrigation treatments. Furthermore, it was observed that the genotypes; Line-1, Line-4, Line-6 and Line-10 recorded the highest values for grain yield and its components under the three irrigation treatments. The findings suggest that these genotypes are reliable promising candidates for developing barley varieties under stress conditions in Egypt.

2.4.3 Related Traits to Drought Tolerance

Breeding for drought-tolerant genotypes based on traits associated with drought tolerance facilitates the selecting process. Some responses have been observed in plants induced by drought stress. The responses have been investigated and associated with morphological and physiological traits (Ludlow and Muchow 1990).

The most important morphological traits in a relationship with drought tolerance in barley are deep and prolific root system, leaf rolling, erect leaves and peduncle length. The deep and prolific root system is a very important adaptive trait for maximizing water uptake and improve productivity under drought stress (Ludlow and Muchow 1990). Additionally, leaf rolling could be considered a drought avoidance mechanism and it is very useful to increase drought tolerance. As, it reduces leaf area and transpiration under water deficit conditions (Clarke 1986; Kadioglu et al. 2012). Erect leaves also allow minimum surface area exposed to the sun which reducing transpiration loss (Sinha and Patil 1986; Reynolds et al. 1999). Similarly, peduncle length could be a useful trait associated with drought tolerance. It was found a strong positive correlation between peduncle length and grain yield under drought stress (Acevedo and Ceccarelli 1989; Kaya et al. 2002).

On the other hand, the physiological traits as leaf cuticular wax, relative water content, proline accumulation and osmotic adjustment are associated with drought tolerance. Various studies showed that leaf cuticular wax could protect the plants against drought where it increases the reflection of solar radiation and reduces water loss (Wójcicka 2015). Relative water content also is a useful indicator to assess the water status of a plant where it associates with physiological activities of plants (Mansour et al. 2020). It is a sensitive variable and quickly responds to environmental conditions, therefore, it could be considered a reliable trait to screen the genotypes for drought tolerance (Rachmilevitch et al. 2016). Moreover, proline accumulation is one of the responses of plants to drought stress (Hanson and Hitz 1982). In drought-tolerant genotypes proline is more synthesized than the sensitive ones under the drought stress (Chandrasekar et al. 2000). Furthermore, the osmotic adjustment is a tolerance mechanism that plants use to cope with drought stress. It refers to a reduction in water potential due to the net accumulation of organic and inorganic solutes as a response to water deficit (González et al. 2008; Nayyar and Walia 2004). This allows the turgor potential to be kept higher and helps limit the effects of water stress on the opening of stomata, photosynthesis and growth (González et al. 2008; Mansour et al. 2020). Besides, carbon isotope discrimination (CID) also correlated positively with grain yield and water use efficiency under drought stress (Farquhar and Richards 1984; Bort et al. 1998; Teulat et al. 2002). Consequently, it could be a useful indicator under water limiting condition.

2.4.4 Genetic Behavior

Drought tolerance represents a complex quantitative trait (polygenic) is governed by multiple loci with multiple components associated with plant water status affecting plant productivity (Al Abdallat et al. 2014; Mir et al. 2012). Accordingly, identification of genetic behavior of drought tolerance and associated traits is important. However, the literature on exploration the genetic control of the related traits to drought tolerance and its mechanism in barley is meager (Madhukar et al. 2018).

Nakhjavan et al. (2009) studied the gene action of some of quantitative traits of barley (plant height, spike length, grain yield in plant, weight of 1000 grain, harvest index, days to heading and days to physiological maturity) under two conditions, normal irrigation and terminal drought. The parents with F_1, F_2, BC_1, and BC_2 generations produced from crosses were evaluated. Results indicated that additive, dominance and epistasis effects presented importance in inheritance of all evaluated traits under normal irrigation and drought conditions. Broad-sense heritability ranged between 0.34 to 0.63 and narrow sense heritability between 0.25 to 0.53 under normal condition. But under terminal drought condition broad and narrow sense heritability varied between 0.48 to 0.77 and 0.29 to 0.62, respectively.

Furthermore, Moustafa (2014) evaluated the type of gene action of grain yield and its components using the six populations (P_1, P_2, F_1, F_2, BC_1 and BC_2) of three barley crosses under two levels of field capacity (50 and 70%). The scaling test provided evidence for the presence of non-allelic interaction for studied traits in all crosses under study. The additive (d), dominance (h) and digenic (additive × additive (i), additive × dominance (j) and dominance × dominance (I)) gene action were significant and involved in the inheritance of most traits under both levels of field capacity.

Likewise, Raikwar (2015) performed generation mean analysis using three crosses to study the nature and magnitude of gene effects for yield and its component traits in barley. The results of this study showed the importance of both additive and non-additive types of gene interaction for all the traits studied. Additionally, the additive × additive (i) type gene interaction and duplicate epistasis also detected suggesting the possibilities of obtaining transgressive segregants in later generations. It was found that the additive × additive (i) epistatic effect was more important and higher than the dominance × dominance (l) effect in the inheritance of a number of effective tillers plant^{-1} and spike length. On the other hand, dominance × dominance epistatic effect was more important in inheritance of grain weight spike^{-1}, 1000-grain weight and grain yield plant^{-1}. Therefore, selection for these traits could be fruitful if delayed to minimize dominance and epistasis effects in the advanced generations.

Besides, Mansour (2017) estimated the type of gene action controlling grain yield and related traits of five barley crosses. These crosses used six-population model to determine the intra and inter-allelic gene interactions controlling the inheritance of related traits in barley. It was found that the dominance × dominance component was greater in magnitude than other components in most traits, indicating that these traits were greatly affected by dominance and its non-allelic interactions.

Similarly, Madhukar et al. (2018) assessed the generation mean analysis for yield and drought related-traits of four barley crosses under rainfed and irrigation conditions. The results suggested that the additive gene effect (d) was more import than the dominance (h) one for 100-grain weight, proline content and plant height. Accordingly, selection for these traits would be effective in the early generations. On the other hand, the dominance (h) gene effect was more important than additive (d) in the inheritance of days to maturity and chlorophyll content. Furthermore, the epistasis was observed for chlorophyll content, stomatal conductivity and grain yield. Also, the dominance × dominance (l) interaction was larger than the additive × additive (i) and additive × dominance (j) effects. Their finding revealed that grain number spike^{-1}, grain weight/spike, and grain yield along with stomatal conductance were predominantly influenced by dominance (h) and dominance × dominance (l) gene action. Therefore, the selection of these traits could be difficult in the early generations and is better to be delayed to advanced generations.

Additionally, Madakemohekar et al. (2018) produced six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) from four different crosses combination in barley to perform generation mean analysis under irrigated and rainfed conditions. The joint scaling test revealed to presence of non-allelic interactions in all four crosses for grain yield and related traits. However, presence of the epistasis effect varied with crosses as well as traits. The generation mean analysis showed that both additive and dominant types of gene effects were important for most studied traits. In general, dominance gene effects (h) were higher than additive gene effects (d) under both irrigated and rainfed conditions. The gene effects indicate that additive component was predominant over dominant for the majority of the traits under rainfed condition. While among the epistatic interaction the major role played by additive × additive type of epistasis which was followed by additive × dominance and dominance × dominance in the four crosses. Which displays that transgressive segregants obtained from these crosses may perform well in the next generations and variety can be developed by selection from these breeding materials.

2.4.5 Breeding and Biotechnology Efforts

Although, classic plant breeding methods have and still present achievement in developing drought-tolerant genotypes, it is a slow process. Therefore, it needs to be integrated with the recent advances breeding methods which improve the process of developing drought-tolerant genotypes (Colmer et al. 2006; Cattivelli et al. 2008; Ashraf 2010). Under Egyptian conditions, several drought-tolerant varieties have been developed of six-row barley, *i.e.* Giza 124, Giza 125, Giza 126, Giza 2000, Giza 132, Giza 133, Giza 134, Giza 137, Giza 138, Giza 129, Giza 130 and Giza 131.

Devolving new barley varieties characterized with drought tolerance depends on generating new allele combinations and subsequent assessing under drought stress then selecting the desirable phenotypes during selfing generations (Nevo 1992). The reliable selection during early generation is difficult due to the heterozygous. Therefore, performing reliable selection requires reaching to acceptable level of homozygosity. On the other hand, the biotechnology tools present rapid achieving homozygosity in barley by producing doubled haploid lines either from immature pollen grains by anther or microspore culture, or through interspecific crosses between barley and *H. bulbosum* with subsequent chromosome elimination (Pickering 1992).

Additionally, mutations induced by radiation or chemical treatments, have also been used for creating genetic variation which could lead to increase crop productivity under drought stress (Parry et al. 2009). Besides, the biotechnology facilitates identification genomic locations of genes controlling traits related to drought tolerance (Lanceras et al. 2004). Detecting stable quantitative trait loci (QTLs) associated with drought tolerance is necessary for utilizing marker-assisted selection (MAS) in breeding. Association of QTLs related to drought tolerance with molecular markers or candidate genes helps breeders to detect the presence or absence of a given QTLs in selected plant material (Collins et al. 2008). QTLs in barley associated with drought tolerance in Mediterranean basin as (Teulat et al. 2001, 2002, 2003; Diab et al. 2004; Wójcik-Jagła et al. 2018).

Marker-assisted selection (MAS) approach is an important method for developing drought-tolerant genotypes not only because of its less time consuming but also labor and cost effective (Kiriga et al. 2016). Furthermore, utilization of MAS allows QTL pyramiding and introducing multiple QTLs related to different drought phenotypic traits into one plant material to improve drought and yield associated traits (Kosova et al. 2014).

The understanding of regulatory networks controlling the drought stress response was enhanced by recent biotechnology tools. This has led to practical approaches for engineering drought tolerance in plants and providing drought tolerant genotypes (Ritala et al. 1994; Umezawa et al. 2006). Genomic analysis has provided gene discovery and allowed genetic engineering using different functional related to drought tolerance in plants (Umezawa et al. 2006). Accordingly, the candidate genes could be transferred to other plant materials during short period compared to classical breeding methods.

2.5 Sunflower

2.5.1 Economic Importance

Sunflower (*Helianthus annuus* L.) has become an important oil crop in the world. The total area reached around 24.8 million hectares worldwide with average productivity of 1.66 ton ha^{-1}. gave total production of 41.3 million metric tons. In Egypt, the total area was about 10,000 hectares with an average production of 2.5 ton ha^{-1} gave total production 25,000 tons (Faostat 2018). The oil has found widespread receipt as a great quality, edible oil, rich in the unsaturated fatty acids, oleic and linoleic acids, vitamin E and contains about 25% proteins.

Egypt suffers from a severe shortage in the production of edible vegetable oils from many problems, leading to a decrease in the local production of oil crops, which led to the failure to meet the needs of domestic consumption (Hassan and Sahfique 2010). Sarvari et al. (2017) reveal that drought stress is a serious adverse factor that limits sunflower growth and productivity. Sunflower is classified as a low to medium drought sensitive crop. The amount and distribution of water have a significant effect on achene and oil yield of sunflower (Krizmanic et al. 2003; Reddy et al. 2003; Iqbal et al. 2005). Conversely, oil quality of sunflower has not been significantly affected by drought condition (Petcu et al. 2001; Pekcan et al. 2015).

2.5.2 Mean Performance and Genetic Diversity

Varietal variances were described for most growth and yield characters (Abou Khadrah et al. 2002; Sharief et al. 2003). Abdel-Wahab et al. (2005) found that among three sunflower hybrids i.e. Euroflour, XF4731 and Vidoc, hybrid XF4731 surpassed the other hybrids for seed yield and oil yield. Sunflower hybrids G-101 and 64-A-93 were the best and most tolerant to drought.. Plant height and dry matter stress tolerance indices for hybrids reduced with increasing drought stress. Whereas an increase in root length stress index was found in the tested sunflower hybrids Dry matter stress index were found to be a reliable indicator of drought tolerance in sunflower (Ahmad et al. 2009). Yasein (2010) verified two sunflower varieties viz. Sakha 53 and Giza 102 under sandy soil conditions and showed that Sakha 53 exceeded Giza 102 in plant height, head diameter, number of seeds/head, seed weight/head, seed yield and seed oil content. Sunflower gave a great genetic variation for osmotic adjustment in response to water shortage (Hussain et al. 2014). Moreover, Pekcan et al. (2015) showed that the differences in sunflower yield varied according to the seasons due to severe heat and drought in the period of growth affected seed yield by 30–35% reduction. Some inbred lines displayed positive responses and some inbred lines attained lost 60% of the control in seed yield. Drought tolerance of male inbred lines in conflict of stress conditions ranged from 50–100% in 1000-seed weight and 70–100% in oil yield. However, oil content of inbred lines was not affected by drought stress; conversely, most of their oil content was increased in a stress environment.

Furthermore, Salem et al. (2013) evaluated 12 sunflower genotypes at three levels of water supply i.e. adequate (7140 m^3 ha^{-1}), moderate (4760 m^3 ha^{-1}) and severe (2380 m^3 ha^{-1}) water regimes. The results demonstrated highly significant differences between the tested genotypes for leaf chlorophyll content, transpiration rate and leaf water content. Combined analyses evidence that L350 had the highest leaf chlorophyll content followed by L460, while the lowest value was recorded by L38. Sunflower genotypes L38 and L20 displayed the highest transpiration rate and L350 was the lowest. Also, the highest leaf water content was recorded by the genotype L11, furthermore L20 and L990 attained higher leaf water content than the remaining genotypes. There was a significant interaction between genotype and the water supply levels for both transpiration rate and the leaf water content. Both transpiration rate and leaf water content decreased significantly for all the genotypes with a reduction in water supply. When the plants received adequate water (7140 m^3 ha^{-1}), the highest transpiration rate was recorded for genotype L11, followed by L38 and L20, whereas L350 attained the lowest transpiration rate. In this treatment, L20 had the highest leaf water content, and Giza 102 and Sakha 53 were the lowest. In the case of moderate drought level, L20 had the highest transpiration rate followed by L38 and L990, while L350 had the lowest value. The highest leaf water content was recorded for L11, though a number of other genotypes had comparable values. The lowest content was found for L38. Under severe drought (2380 m^3 ha^{-1}), L8 had the highest transpiration rate followed by L990 and L11, while Sakha 53 and L460 had the lowest rates. The highest leaf water content was recorded for genotype L11, followed by L350, which was on par with several other genotypes. With an adequate water regime, L990 produced the highest seed yield, while L11 was the lowest. In the case of both moderate and severe drought, L38 had the highest seed yield, while L350 and L11 were the lowest ones. The interaction between genotypes and water regimes had a significant effect on the seed and oil yields. When the plants were provided with an adequate water regime, L990 had the highest oil yield, on par with L8, L460 and Giza 102, while L11 had the lowest. In the case of moderate or severe drought, L38 produced the highest oil yield, followed by L20, L460 and Giza 102 on par at both levels, together with L235 and L8 in the severe water regime. Genotypes L350 and L11 gave the lowest oil yield at both moderate and severe water supply levels.

2.5.3 Related Traits to Drought Tolerance

Acclimation of plants to water stress is due to different traits and the occurrence of adaptive changes in morphological, physiological and biochemical properties such as changes in plant construction, growth, osmotic adjustment and antioxidant defenses

(Duan et al. 2007). Evolution in plant protection systems occurs through the production of antioxidants including enzymatic and non-enzymatic protection to reduce oxidation from ROS (Davar et al. 2013). Scavenging of Reactive oxygen species ROS and decreasing their damaging effects might associate with drought tolerance of crops. The elimination of ROS requires the activity of many enzymes such as APX1, CAT2, SOD3, POX4, MDHAR5, DHAR6, GR7 as well as non-enzymatic i.e. ascorbate, phenolic compounds, carotenoids, glutathione, glycine betaine, proline, sugar, polyamines (Gill and Tuteja 2010; Karuppanapandian et al. 2011).

Sarvari et al. (2017) assessed physio-biochemical changes and antioxidant enzymes activities of six sunflower lines under normal and irrigation at 60 and 40% of the field capacity. They recorded significant differences among sunflower lines for physio-biochemical and enzymes activity under drought stress condition. Genotypes C104 and RHA266 were best tolerant to drought stress. The resistant genotypes had a better osmotic adjustment to maintain their water potential under drought and normal situations. The highest relative water content was correlated with genotypes LR55 and RHA266 at 60% and the lines C104 and RHA266 at 40% drought stress. Genotype LR25 showed the highest rise in chlorophyll under 60% and the genotype RHA266 under 40% drought stress. The highest increase in carotenoid content was registered in the genotype C100 at 60% and the genotype RHA266 at 40% drought stress. The genotype C100 had the highest carotenoid content in comparison with the others under both drought stress conditions. The highest proline content was recorded in genotypes C104 and LR25 at 60% and 40% drought stress, respectively. The lowest values of malon dialdehyde belonged to the genotypes LR4 and RHA266 under 60% drought, however genotype LR55 exhibited lowest values at 40% drought stress. Genotype C100 gave the highest catalase activity at 60% and in genotypes C104 and RHA266 at 40% drought stress. Genotypes C104 and RHA266 had well water status and osmotic adjustment and tolerate to drought stress. The highest glutathione reductase activity was detected in genotypes C100 and RHA266 at 60% and genotype C104 at 40% drought stress. Both genotypes C100 and LR4 gave the highest ascorbate peroxidase activity at 60 and 40% drought stress, respectively. The highest guaiacol peroxidase activity belonged to genotypes LR25 and LR55 under 60 and 40% of drought stress, respectively. Santhosh (2014) showed that sunflower genotypes DRSF-113 and EC-602063 recorded better values for morphological, physiological and yield characters over other genotypes under both moisture stress and control situations. Genotypes DRSF113 and EC-602063 showed better tolerance under stress conditions. The different plant features helps to maintain plant water status or enable the plant to tolerate stress like root traits, leaf area, leaf water potential, photosynthesis, growth rates and rapid recovery after stress, water use efficiency, and biochemical traits such as chlorophyll and membrane stability, proline, osmoregulation and protecting enzymes.

Results of correlation revealed the importance of transpiration rate, plant height, 100-achene weight and leaf water content as selection criteria for improvement of sunflower yield under normal condition. While, under water-stressed state, head weight, head diameter, number of achene and chlorophyll content showed positive and significant correlation with seed yield plant^{-1}. Head diameter and number of achene under both conditions and chlorophyll content under water-stressed condition have a positive direct effect on seed yield plant^{-1}. Also results based on path coefficient analysis recorded by Awaad et al. (2016) showed that maximum direct effect on achene yield plant^{-1} was accounted for transpiration rate and plant height with values of 12.941% and 12.219%. Whereas, moderate direct effects were recorded by both 100-achene weight and leaf water content with values of 7.128 and 7.779%. Moreover, the other remaining three characters, i.e., leaf chlorophyll content, achene oil content and head diameter had less contributions as exhibited 1.917, 0.438 and 0.081%, respectively. The highest indirect effects on achene yield plant^{-1} variation were observed for transpiration rate via plant height followed by transpiration rate via 100-achene weight, leaf water content via 100-achene weight, plant height via 100-achen and leaf chlorophyll content via plant height with values of 8.442, 5.530, 4.579, 3.181 and 2.858, respectively (Table 2.5). According to the total contribution of the studied characters on achene yield/plant variation, it could be arranged as follows, transpiration rate (22.778%), plant height (20.413%), 100-achene weight (13.939%), leaf water content (12.796%), leaf chlorophyll content (4.997%), achene oil content (2.571%) and then head diameter (0.871%). Generally, it could be concluded that the studied characters accounted for 78.365% of the achene yield/plant variation. However, the residual effect was 21.635%.

2.5.4 Genetic Behavior

The study of genetic behavior is important before starting the implementation of the breeding program to choose suitable breeding method to improve the economic characteristics in sunflower. Ortis et al. (2005) indicated the predominant role of the additive gene action in controlling plant height,1000—achene weight and seed oil content of sunflower. Whereas, Mijic et al. (2008) showed that general combining ability variance was larger than specific combining ability for achene yield, oil content and oil yield. Moreover, general combining ability variance was greater than specific combining ability variance for yield, head diameter and oil content (Machikowa et al. 2011). 1000-achene weight, achene number head^{-1} and oil yield were inherited under both additive and dominant effects. Plant height and oil content were governed by additive effects, however over dominant effect was noticed for achene yield (Ghaffari and Mirzapour 2011).

Salem and Ali (2012) aimed to select sunflower parents with good general combining ability (GCA) and crosses with best specific combining ability (SCA) effects under different levels of water supply i.e. adequate (7140 m^3 ha^{-1}), moderate (4760 m^3 ha^{-1}) and severe (2380 m^3 ha^{-1}). Seven sunflower inbred lines were crossed in a 7 × 7 half diallel cross fashion. The resultant 21 hybrids were evaluated along with their parents to yield contributing characters and oil content. Mean squares of genotype x environment interactions were highly significant for plant height, transpiration rate, yield and oil content, suggesting the differential response of sunflower genotypes to water stress environments. The variance due to general combining

Table 2.5 Direct and indirect effect of various metric traits of sunflower genotypes on achene yield plant^{-1} across three environments (Awaad et al. 2016)

S.O.V.	CD	RI %	Total contribution on achene yield plant^{-1}
Leaf water content % (X_1)	0.07779	7.779	12.796
Leaf chlorophyll content (X_2)	0.01917	1.917	4.997
Transpiration rate (X_3)	0.12941	12.941	22.778
Plant height (X_4)	0.12219	12.219	20.413
Head diameter (X_5)	0.00081	0.081	0.871
100-achene weight (X_6)	0.07128	7.128	13.939
Achene oil content % (X_7)	0.00438	0.438	2.571
X_1 x X_2	0.01746	1.746	
X_1 x X_3	0.02367	2.367	
X_1 x X_4	0.00537	0.537	
X_1 x X_5	0.00056	0.056	
X_1 x X_6	0.04579	4.579	
X_1 x X_7	0.00748	0.748	
X_2 x X_3	0.01270	1.270	
X_2 x X_4	0.02858	2.858	
X_2 x X_5	0.00101	0.101	
X_2 x X_6	0.00525	0.525	
X_2 x X_7	0.00132	0.132	
X_3 x X_4	0.08422	8.422	
X_3 x X_5	0.00615	0.615	
X_3 x X_6	0.05530	5.530	
X_3 x X_7	0.01470	1.470	
X_4 x X_5	0.00678	0.678	
X_4 x X_6	0.03181	3.181	
X_4 x X_7	0.00713	0.713	
X_5 x X_6	0.00074	0.074	
X_5 x X_7	0.00055	0.055	
X_6 x X_7	0.00207	0.207	
R^2	0.78365	78.365	78.365
Residual	0.21635	21.635	21.635
Total	1.00000	100	100.000

ability was greater than that of specific combining ability for the studied characters, indicating the preponderance of additive gene action. The general combining ability effects of the parents revealed that the inbred lines L350, L460, L990 and L770 proved to be good combiners for achene yield, while the parents L38, L990 and L235 were found to be promising general combiners for oil % content. Inbred lines L38, L11 and L235 were good candidates for drought tolerance. L350 was a good combiner for seed yield, while L38 proved to be good combiner for oil content and drought tolerance. On the basis of SCA, the cross L38 x L350 was identified as promising seed yield and oil content. On the other hand, Awaad et al. (2016) assessed heterosis as a percentage of mid-parents for leaf water content, leaf chlorophyll content and transpiration rate of sunflower. Cross combinations P_1 x P_2, P_1 x P_6, P_4 x P_5 and P_4 x P_6 gave significant positive heterotic effects for leaf water content at adequate water supply. Meanwhile, no significant positive heterosis was found among all crosses at both moderate and severe stress treatments. Also, significant positive heterosis was found for leaf chlorophyll content in the F_1 crosses P_1 x P_2 and P_4 x P_6 at adequate water supply; P_1 x P_3, P_2 x P_3, P_2 x P_7 and P_3 x P_7 at moderate and P_2 x P_3 and P_3 x P_4 at severe drought stress as presented in Table 2.6. On the other hand, desirable significantly negative heterosis has been registered for transpiration rate by only one cross (P_5 x P_6) at adequate water supply; P_1 x P_7, P_2 x P_3, P_2 x P_4, P_2 x P_5, P_2 x P_7, P_3 x P_4, P_3 x P_5, P_3 x P_7, P_5 x P_6 and P_5 x P_7 at moderate and all crosses at severe stress (Salem and Ali 2012). These results were desired for the breeder where the genotypes, which maintain high levels of leaf water content with high ability to reduce transpiration rate might be considered as tolerant to water stress. It is interesting to note that the sunflower cross ($P_1 \times P_6$) produced the maximum value of heterosis for achene yield plant^{-1} under adequate water supply (22.77%) and moderate (22.58%) level with DSI value less than 1.0 and had significantly negative heterosis for plant height (−10.52%) and the transpiration rate (−44.93%) at severe stress. Therefore it could be considered the promising one and classified as tolerant to water stress.

2.5.5 Breeding Efforts and Biotechnology

Drought can be managed by modifying the plant morphology or incorporating required attributes that help the plants to mitigate the effect of drought stress effectively (Yordanov et al. 2000). Thus, genetic modification is the most successful and cheapest strategy to deal with drought. Because modifications in plant morphology and physiology are heritable, then transferred into breeding materials will be a permanent source of drought tolerance. There are several breeding strategies for drought stress tolerance in crop plants like induction of earliness for drought escape, modification of some plant traits related to drought tolerance and the introduction of traits associated with high yield. Strategy for breeding drought stress depends upon the intensity, frequency and timing of drought occurrence. Also, mutations affecting seed oil fatty acid composition are very useful for getting a novel oil composition (Lacombe et al. 2000). The presence of mutant genes to increase levels of individual

Table 2.6 Heterosis over mid-parent (M.P.) for physiological characters of sunflower crosses of half-diallel analysis in three environments (Awaad et al. 2016)

Character Water supply Genotype	Leaf water content (%)			Leaf chlorophyll content (SPAD)			Transpiration rate (mg $H_2O/cm^2/h$)		
	Adequate water supply	Moderate stress	Severe stress	Adequate water supply	Moderate stress	Severe stress	Adequate water supply	Moderate stress	Severe stress
P1 x P2	5.51^{**}	−3.67	-8.99^{**}	4.97^{*}	6.43	3.97	10.00^{**}	−7.69	-41.25^{**}
P1 x P3	0.51	−2.50	-9.82^{**}	0.00	7.81^{*}	1.60	3.75	−7.26	-45.07^{**}
P1 x P4	2.35	−3.27	-6.24^{**}	−0.10	0.83	1.56	10.98^{**}	−1.47	-23.53^{**}
P1 x P5	1.74	−5.73	-9.63^{**}	3.06	6.72	−1.48	8.16^{*}	1.33	-42.11^{**}
P1 x P6	3.66^{*}	−5.08	-5.64^{*}	0.57	4.42	−0.28	8.33^{*}	3.13	-44.93^{**}
P1 x P7	−1.86	−4.53	-7.77^{**}	−2.47	7.34	−4.96	8.24^{*}	-20.19^{**}	-55.00^{**}
P2 x P3	0.84	0.65	-12.91^{**}	−0.03	11.80^{**}	8.12^{*}	2.17	-13.57^{**}	-53.57^{**}
P2 x P4	1.79	−0.06	-15.09^{**}	1.11	0.62	3.49	2.27	-15.49^{**}	-40.74^{**}
P2 x P5	0.19	2.21	-11.69^{**}	−0.56	5.41	4.29	−0.98	-11.54^{**}	-55.81^{**}
P2 x P6	2.07	0.23	-10.66^{**}	2.10	4.83	1.49	3.81	−6.47	-50.00^{**}
P2 x P7	−1.05	−2.58	-12.92^{**}	−2.54	8.84^{*}	−2.48	2.15	-25.83^{**}	-70.00^{**}
P3 x P4	2.05	−5.41	-15.14^{**}	3.85	4.09	8.23^{*}	2.38	-11.43^{**}	-44.00^{**}
P3 x P5	−0.73	1.68	-16.97^{**}	−0.69	7.49	1.07	0.00	-9.21^{*}	-65.79^{**}
P3 x P6	0.01	0.29	-18.51^{**}	1.42	−0.73	2.55	−1.04	−2.67	-73.53^{**}
P3 x P7	−2.13	-6.81^{*}	-18.10^{**}	−2.81	12.58^{**}	−1.11	2.25	-10.00^{**}	-73.44^{**}
P4 x P5	3.24^{*}	0.13	-15.68^{**}	−4.66	−1.08	−2.67	0.00	−3.95	-21.65^{**}
P4 x P6	3.54^{*}	−4.24	-10.95^{**}	6.57^{*}	−3.44	2.21	−3.33	−0.61	-22.22^{**}
P4 x P7	2.52	−3.81	-10.20^{**}	-8.74^{*}	−5.46	−1.37	2.35	−8.87	-28.75^{**}
P5 x P6	−0.49	−4.60	-25.21^{**}	−3.54	2.42	1.73	-10.00^{**}	-19.88^{**}	-105.71^{**}
P5 x P7	1.69	−3.97	-18.51^{**}	0.07	4.21	−3.10	−1.01	-17.36^{**}	-114.06^{**}

(continued)

Table 2.6 (continued)

Character	Leaf water content (%)			Leaf chlorophyll content (SPAD)			Transpiration rate (mg $H_2O/cm^2/h$)		
Water supply Genotype	Adequate water supply	Moderate stress	Severe stress	Adequate water supply	Moderate stress	Severe stress	Adequate water supply	Moderate stress	Severe stress
P6 x P7	0.75	−6.35	−15.87**	−0.34	2.16	−2.58	3.92	−7.14	−121.67**

*,** Significant at $P = 0.05$ and $P = 0.01$, respectively fatty acids and for different forms and levels of tocopherol leads to improved oil quality and composition of sunflower hybrids (Skoric et al. 2008).

Consequently, the genetic improvement of plant cultivars for water stress tolerance and the ability of a crop plant to produce maximum yield over a wide range of stress and non-stress conditions has been a principal objective of breeding programs for a long time (Chachar et al. 2016; Moustafa et al. 1996). Some tolerant genes were determined in wild types and transferred into cultural ones (Kaya 2014). On the other hand, especially in controlled conditions, sunflower genotypes might be screened to produce tolerant lines to drought stress with simultaneous selection (Geetha et al. 2012; Soorninia et al. 2012). Under Egyptian conditions, drought-tolerant varieties have been developed through breeding programs such as Sakha 53 and Giza 102.

Marker-assisted selection approach serves the purpose of efficient transfer of the desirable gene into elite sunflower inbreds which infers sustainable saving in time compared to conventional backcross breeding. Markers/QTLs would be useful in the marker-assisted selection in breeding program to improve oil quality (Premnath et al. 2016). Molecular such as RAPD and ISSR and the biochemical like protein markers were used to assess the genetic relationships among genotypes. In this respect, based on genetic similarity using the Dice coefficient, Abdel-Ghany (2012) studied morphological traits, oil content and fatty acids of six sunflower genotypes. Genotypes showed insignificant differences among them except for plant height and 1000-seed weights. Bani Suef, Aswan and Beharia genotypes exhibited a high proportion of polyunsaturated fatty acids. Molecular markers results revealed that the genetic similarity ranged from 9% to 77% and 24.1% to 96.6% for the RAPD and ISSR, respectively. Cluster analysis based on similarity matrices of ISSR and combined data separated Giza 102 from all the other genotypes, while the other genotypes fall in a second cluster. RAPD produced 29 unique markers for five of the six genotypes. However, ISSR technology has revealed the ability to discriminate 22 unique markers in the four sunflower genotypes from the six genotypes under study. The biochemical analysis showed slight differences among the six genotypes. Furthermore, biotechnological techniques based on tissue culture were utilized to define the best genotypes and the best explant suit different genotypes. Different regeneration media with different concentrations of BA and NAA showed that the shoot tips of developing the highest rate of response and El-wadi El-gidid genotypes showed the best ones for shoot formation in different concentration of hormones rather than the other genotypes.

Ali et al. (2018) estimated a number of morphological characters, along with relative water content in 100 inbred sunflower lines under natural and water stress during two successive years. At the molecular level, 30 simple sequence repeat (SSR) primer pairs and 14 inter-retrotransposon amplified polymorphism (IRAP) also14 retrotransposon-microsatellite amplified polymorphism (REMAP) primer combinations were used for DNA fingerprinting of the genotypes. Most of the studied characters exhibited lower values under water stress than natural states. Maximum and minimum decreases were observed in yield and oil percentage. Broad-sense heritability for the examined characters ranged from 0.20–0.73 and 0.10–0.34 under natural and water stress states, respectively. In the verified samples, 8.97% of the

435 possible locus pairs of the SSRs represented significant linkage disequilibrium levels. The SSR analysis identified 22 and 21 markers for the studied characters under natural and water stress states, respectively. The corresponding values were 50 and 37 using retrotransposon-based molecular markers. Some detected markers were common among the characters under water stress and natural states.

2.6 Conclusions

Based on the above discussions, we can state the following conclusions:

1. Drought stress has significant negative effect on plant growth, yield components, grain quality and yield of crop plants, particularly under current climate change.
2. Drought-tolerant genotypes produce considerably greater yield compared with drought-sensitive genotypes especially under water deficit conditions. Therefore, utilizing drought-tolerant genotypes is essential to increase productivity particularly in arid and semi-arid regions in the Mediterranean.
3. There are various morphological and physiological traits associated with drought tolerance could be exploited in selecting drought-tolerant genotypes; as deep and prolific root system, leaf rolling, erect leaves, peduncle length, leaf cuticular wax, relative water content, proline accumulation, osmotic adjustment and antioxidants.
4. Integration of recent advances breeding methods (as quantitative trait loci, Marker-assisted selection and genetic engineering technique with classic plant breeding help significantly in developing drought-tolerant genotypes accurately and rapidly.

2.7 Recommendations

The chapter highlights the following recommendation:

1. Combining morpho-physiological and biochemical traits can provide a more complete model of gene-to-phenotype relationships and genotype-by-environment interactions to tolerate drought stress.
2. Integration of recent improvement procedures as DNA-marker assisted selection and gene manipulation with classic plant breeding aids in improving drought-tolerant genotypes accurately and rapidly.

Acknowledgements Hassan Awaad and Elsayed Mansour acknowledge the partial support of the Science and Technology Development Fund (STDF) of Egypt in the framework of the grant no. 30771 for the project titled "A Novel Standalone Solar-Driven Agriculture Greenhouse - Desalination System: That Grows Its Energy And Irrigation Water" Via The Newton-Musharafa Funding Scheme.

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