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Response of Chickpea (*Cicer arietinum L.*) Genotypes to Drought Stress at Different Growth Stages

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Abstract**—**Chickpea (*Cicer arietinum* L.) is one of the important grain legume crops in the world. However, drought stress is a serious threat to chickpea production, and development of drought-resistant varieties is a necessity. Field experiments were conducted to evaluate the response of 8 chickpea genotypes (MCC* 696, 537, 80, 283, 392, 361, 252, 397) and drought stress (S1: non-stress, S2: stress at vegetative growth stage, S3: stress at early bloom, S4: stress at early pod visible) at different growth stages. Experiment was arranged in split plot design with four replications. Difference among the drought stress time was found to be significant for investigated traits except biological yield. Differences were observed for genotypes in flowering time, pod information time, physiological maturation time and yield. Plant height reduced due to drought stress in vegetative growth stage. Stem dry weight reduced due to drought stress in pod visibly. Flowering time, maturation time, pod number, number of seed per plant and yield cause of drought stress in flowering was also reduced. The correlation between yield and number of seed per plant and biological yield was positive. The MCC283 and MCC696 were the high-tolerance genotypes. These results demonstrated that drought stress delayed phonological growth in chickpea and that flowering stage is sensitive.

Keywords**—**Chickpea, drought stress, growth stage, tolerance.

I. Introduction

THE chickpea (*Cicer arientinum* L.) is a self-pollinated and diploid (2n=2x=16) plant whose origin is East Turkey, but its cultivation spread to India and Europe [1]. This crop is grown in a wide range of climatic conditions from sub-tropical regions of the Indian subcontinent and North-eastern Australia to the Mediterranean regions of West Asia, North Africa, South and Southwest Europe [2]. Chickpea is one of the most important food crops and amongst one-year-grain crops, it has the 14th rank in terms of acreage area and it has the 16th rank in terms of production, [3]. Chickpea is not only an important source in the human diet, but also plays an important role in biological nitrogen fixation in the soil. There are two main types of chickpea crop, Desi and Kabuli. Desi is assigned approximately 85% of its acreage and mainly in West Asia and Iran, Ethiopia and Australia while Kabuli is mainly growing in Mediterranean countries, North Africa and North America [4].

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Biotic and abiotic stresses, are limiting factors for the yield in many parts of the world and that is why significant differences in yield in different areas can be observed. This plant is mainly cultivated by smallholder farmers in arid and semi-arid areas, where they often face a lack of rainfall and irrigation water [5]. Drought is a meteorological term and an environmental event, defined as a water stress due to lack or insufficient rainfall and/or inadequate water supply [6]. Decreased yield due to drought stress has varying degrees and depends on time, intensity, and stress time, while it is influenced by other stressful environmental factors such as temperature and light [7]. Normally chickpea is grown under rainfed condition and at the end of the rainy season to be grown by using the residual moisture in the soil and the least rainfall at development time, in major chickpea producer countries such as India (9880000 tones), Pakistan (750000 tones) Turkey (450000 tones) and Iran (275310 tones), most cultivation acreage is in arid and semi-arid regions [8]. In these areas, drought stress occurred during the growing season and in the final two groups. The final stress caused by reduced soil moisture in pod and seed filling stages is of greatest concerns of chickpea production in these areas [9]. Therefore, increased chickpea production under drought conditions depends on their compatibility development [2]. The purpose of the tests in both stress and non-stress conditions is selection of genotypes compatible with both environments which are evaluated with different indicators. Because with once evaluation of genotypes with different indicators, more accurate grouping is possible for them. According to the results reported by different researchers, figures are favorable and stable which give the best answer in both conditions [10]. Various methods are used to apply the stress on the farm including irrigation stoppage at different stages of plant growth, simulation of drought at the beginning of the season, at the mid-season and at the end of season to measure plant response to drought at different stages. In addition, there is the possibility of monitoring the plant recycling after re-watering at any stage. So, having excellent relative performance of genotypes, is a common starting point to achieve genotypes with partial resistance and their selection in the phonological stages of growth and development under stress conditions and to achieve that, understanding plant reactions and behaviors in the face of drought is necessary, in this study, the effect of drought stress in different growth stages of chickpea on yield and morphological characteristics of the plant has been investigated.

II. Materials and Methods

To evaluate the response to drought stress, 8 superior genotypes of chickpea (MCC696, 537, 80, 283, 392, 361, 252, 397) which have shown better resistance and tolerance in the researches at the Mashhad Institute of Plant Sciences tests, were selected [11]. Test was conducted in the spring of 2013 in the farms of agriculture and natural resources research center of North Khorasan, Iran. Effects of drought treatment were studied at four levels, (S1: non-stress, S2: stress at vegetative growth stage, S3: stress at early bloom, S4: stress at early pod visible) in an experimental design as split plot in a completely randomized block design with four replications where drought stress was considered as main factor and genotypes were considered as sub-factor. Each experimental plot consisted of four rows of six meters where row spacing was 50 cm and plants distances on row was 10 cm with the density of 20 plants per square meter and 75 cm distance between plots from each other. Land preparation including deep autumn plowing, spring plowing and disc and cultivation in May, according to local custom were conducted. For each of the levels of stress with the onset of associated phonological stage the irrigation has been cut until the soil water reaches to 20 percent of field capacity so that the symptoms of stress including closing leaves and drooping mode in the terminal bud were showed. Practices including weeding, controlling pest and plant diseases exactly were done during the growing season. The parameters measured were the time of flowering (flowering 50% of the plants), maturity (maturity 50% of plants), plant height, number of pods and number of seeds per plant,100 seed weight, shoot dry weight, grain yield and biological yield per plant. The analysis of the results and graph drawing were conducted using software's JMP, Mstat-C and Excel. All parameters measured during tests were defined based on a single plant. Therefore, all data given in the paper are related to a single plant.

III. Results and Discussion:

Based on the results of analysis of variance, the drought stress impact on plant height in the corresponding phonological stages of growth was significant ($P<0.05$) (Table I). The highest plant height of 54.71 cm was associated with control and the lowest plant height of 49.9 cm height is related to the stress treatment at vegetative stage. Based on the results of averages comparison, plant height reduction was significant in the growth stage than the control subject, but it is not significantly different from two other stages of stress applying (Fig. 1) therefore, it seems stress at vegetative stage, impressed the plant height as after removing the stress, continuity of growth failed to offset this impact and the plant height has not been improved, on the other hand at reproductive growth stage, most of photosynthetic materials are allocated to reproductive organs, therefore the plant height at this stage are less affected by stress, Because the growth phenomenon of vital activities in the conditions that plants must have enough water, if there is no water supply required due to reduced Turgor pressure of growing cells and the effect on the cells, the height will be reduced [12].

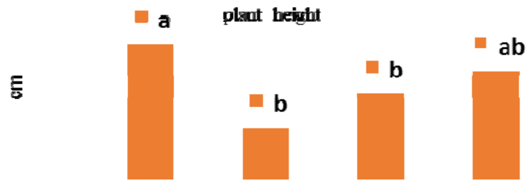

Fig. 1 Drought stresses stages

The investigation of correlation coefficients between traits shows that the highest positive correlation is between plant heights and shoots dry weight and equal to 0.46. Increase in plant height has a significant positive correlation with two important functional components of the pods and seeds number; therefore, in the case of decrease in plant height, it will affect the reduction in yield through these two traits. In addition, there has been a negative correlation with grain weight which showed that the relationship is negative. A positive correlation between plant height and biological performance can have a positive impact on the performance and for this reason; perhaps it is more useful to select tall varieties [13]. There are also significant differences in plant height between genotypes, which are caused by differences in their genetic potential (Table I). MCC283 genotype had the highest plant height and genotype CC392 most had the lowest plant height with the values of 56.5 cm and 47.36 cm (Table II). Moreover, interaction of drought stress × genotypes is also significant, which represent the different behavior of genotypes under stress and non-stress conditions. Stress impact at different phonological stages of the plant's shoot dry weight was significant (Table I). Control treatment had the highest shoot dry weight (19.53 gr per plant) and stress at pod filling stage (18.65gr per plant) had the lowest shoot dry weight. Statistically, between stress treatments at flowering and pod filling stages no significant difference was observed in terms of shoot dry weight (Fig. 2). So, it can be said that in different reproductive stages, stress will have similar effect on the final dry matter. Because stress causes severe loss of leaves and the plant growth cannot do the necessary recovery.

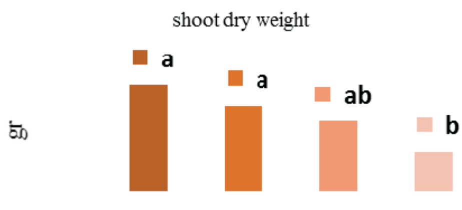

Fig. 2 Drought stresses stages

Chickpea under drought stress conditions decreased development of vegetative organs to reduce the level of photosynthesis and their photosynthetic energy and materials are corresponded to reproductive growth to protect the survival. Positive correlation between these traits and plant height proves that under drought stress conditions plant height and numbers of leaves are reduced, as a result, shoot dry weight is also reduced. In studies on a variety of plants, descending trend of shoot dry weight is reported in more negative potentials [14]. Genotypes tested had significant differences in terms of shoot dry weight (Table I). Among them, genotype MCC283 (20.8 gr) and MCC397 (18 gr) showed the highest and the lowest shoot dry weight, respectively. Also the interaction of genotype × stress is not significant that indicates the uniform impressionability of all genotypes by applying stress on phonological stages of chickpea, so perhaps genotypes with high dry matter production under drought stress conditions can be introduced as tolerant genotypes. Significant positive correlation (0.54) between biological yield and shoot dry weight reflects the deep relation between these two traits with genotype plants. Biological yield has shown a significant positive correlation (0.67) with the number of grains per plant and number of pods per plant (0.47). Other researchers have also reported significant correlation between grain yield and dry matter production in the stress condition [15].

The effect of stress at various phonological stages on both traits of the number of days to flowering and days to maturity was significant (Table I). Flowering stage in both the trait was most affected by the drought, but statistically significant difference was observed between stress treatments during vegetative growth and flowering stages in terms of the number of days to flowering and maturity (Figs. 3 and 4).

One of the effects of stress was to put forward the flowering stage and totally acceleration of phonological process that is a sort of escape from drought, so it seems that middle drought stress causes early matured chickpeas which will consequently reduce the yield. This reduction will be through the numbers of pods and seeds per pod. Negative correlation between days to maturity, number of pods (-0.23) and number of seeds per plant (-0.20) confirms this fact.

There was a significant difference between genotypes tested in terms of time of flowering and maturity. MCC80 was the most precocious with 52 days and MCC261 was the latest genotype with 61 days (Table II). Significant genetic variation among chickpea genotypes for days to flowering has also been reported by other researchers. These researchers suggested short days to flowering as a useful trait for areas with extreme drought conditions. These results confirm the findings of other researchers in conjunction with more impact of genotype on the flowering date of chickpea compared to moisture regime [16]. In many chickpea genotypes, days to flowering is a function of temperature and only in a few numbers of genotypes days to flowering is controlled by photoperiod. According to the researchers, the correlation between days to flowering and days to maturity ($r = 0.6017$) was positive and significant [17].

Other researchers reported a high positive correlation ($r = 0.44$) between days to flowering and days to maturity in chickpea genotypes. The optimal interval for days to flowering is a major component of plant adaptation to environmental conditions and it is a critical trait for plant adaptation to specific latitude. Clearly, over precociousness will result in yield reduction. Days to flowering is a quantitative trait controlled by multiple genes, but a large gene as a gene responsible for a high diversity available for this trait among chickpea genotypes has been reported [18].

According to the findings of [17], flowering genes are effective on days to maturity by affecting the reproductive growth and then, durability of reproductive growth stage.

Therefore, most of the improvements in performance and stability is probably due to the combined effect of duration of vegetative growth and limited soil water availability, as in chickpea the correlation between the number of days to flowering and performance implies that perhaps in later years, if the soil moisture conditions were above average, the yield is still at a low level. Therefore, it has been advised to selection for unlimited growth habit along with early flowering that has increased the timing of flowering and pod filling, leading to an increase in crop yield.

Effects of drought stress the number of pods per plant at phonological stages of growth has been significant (Table I). The highest number of pods per plant was associated with control group and the lowest number of pods per plant was related to the treatment of stress at flowering growth stage (Fig. 5). There was a significant difference in the number of pods per plant between genotypes. Among them MCC283 and 397 MCC had the most and the lowest number of pods per plant, respectively (Table II). Effect of drought stress on the number of seeds per plant at growth stages has been significant (Table I). The greatest number of seeds per plant with 126 numbers was associated with the controls and the lowest number of seeds per plant was related to stress treatment with 117 numbers at flowering stage.

Based on the results of comparison of average values, reduced number of seeds per plant in flowering stage is significant compared to the controls between genotypes tested there was a significant difference in the number of seeds per plant.

Among them MCC283 and 397 MCC had the most and the lowest number of seeds per plant, respectively. At the same time these two genotypes also had the highest and lowest yield (Table II).

Data analysis of many field trials has shown that the late flowering stage is the most sensitive stage to drought, high sensitivity in the reproductive stage, perhaps is because of the aging of a number of primary roots and the lack growth of new roots at this stage [19], [20].

The number of seeds per plant that is an important component of performance that has shown significant positive correlation with the number of pods (0.688) and a negative correlation with seed weight (-0.492) therefore, it seems that in the process of selection of desirable genotypes to achieve varieties tolerant to drought, the resultant changes under stress conditions is very important. Because the initial water stress in pod growth stage of crops, affect their number. While the stress affect the number of seeds per pod at later time [21].

TABLE I
MEANS SQUARES OF INVESTIGATED CHARACTERISTICS IN CHICKPEA GENOTYPES UNDER DROUGHT STRESSES

S.O.V	D.f	Day to flowering	Day to maturity	plant height	shoot dry weight	number of pods per plant	number of seeds per plant	100 seed weight	Biological yield Per plant	Seed yield Per plant
Replication	3	77.133**	77.133**	107.663*	0.143^{ns}	143.28^{ns}	40.257^{ns}	0.535^{ns}	17.200^{ns}	22.06^{ns}
Drought stresses	3	24.320**	24.320**	124.906*	4.403*	898.244*	462.575*	20.433**	42.856^{ns}	90.813*
Error $_a$	9	2.452	2.452	22.218	0.956	222.280	113.743	1.092	52.562	19.190
Genotypes	7	128.427**	128.427**	200.828**	13.838**	1340.090**	1178.963**	309.099**	730.593**	107.09*
Error×Genotypes	21	1.731^{ns}	1.731^{ns}	33.683*	1.570^{ns}	183.938^{ns}	136.990^{ns}	5.459*	138.678^{ns}	31.32**
Error$_b$	84	3.801	3.801	17.591	1.345	146.398	133.749	3.243	95.661	10.558
C.V	-	3.39	3.39	8.04	6.07	11.27	9.45	6.54	10.17	9.31

TABLE II
EFFECT OF DROUGHT STRESS ON INVESTIGATED CHARACTERISTICS IN CHICKPEA GENOTYPES

Genotypes	day to flowering	day to maturity	plant height	shoot dry weight	number of pods per plant	number of seeds per plant	100 seed weight	biological yield per plant	Seed yield per plant
MCC696	39.50^{de}	58.38^{b}	56.22^{a}	18.40^{cd}	117.4^{cd}	107.4^{cd}	88.79^{cd}	88.79^{cd}	.3626^{a-c}*
MCC252	41.75^{bc}	56.75^{cd}	52.91^{b}	19.73^{b}	124.4^{bc}	111.4^{bc}	94.41^{bc}	94.41^{bc}	30.96^{d}
MCC537	42.31^{b}	58.75^{b}	52.48^{b}	19.26^{b}	117.7^{cd}	99.75^{de}	97.78^{ab}	97.78^{ab}	35.79^{bc}
MCC80	37.94^{e}	52.13^{e}	54.38^{ab}	19.13^{bc}	131.5^{ab}	116.9^{ab}	100.9^{ab}	100.9^{ab}	34.03^{c}
MCC392	40.44^{cd}	55.44^{d}	47.36^{c}	19.37^{b}	114.8^{d}	100.1^{de}	104.0^{a}	104.0^{a}	36.60^{ab}
MCC283	43.06^{b}	57.94^{bc}	56.50^{a}	20.80^{a}	137.3^{a}	121.3^{a}	103.2^{a}	103.2^{a}	38.40^{a}
MCC361	45.50^{a}	60.88^{a}	48.05^{c}	18.12^{d}	124.0^{bc}	108.2^{cd}	95.06^{bc}	95.06^{bc}	31.40^{d}
MCC397	44.81^{a}	60.44^{a}	49.52^{c}	18.00^{d}	112.4^{d}	94.13^{e}	85.03^{d}	85.03^{d}	35.64^{bc}
LSD$_{(0.05)}$	1.636	1.371	2.949	0.8154	8.507	8.131	1.266	6.877	2.285

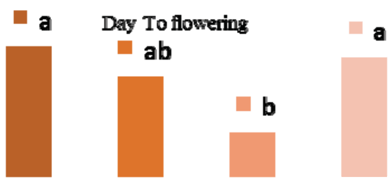

Fig. 3 Drought stresses stages

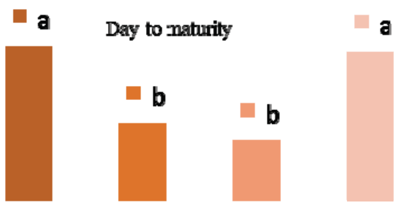

Fig. 4 Drought stresses stages

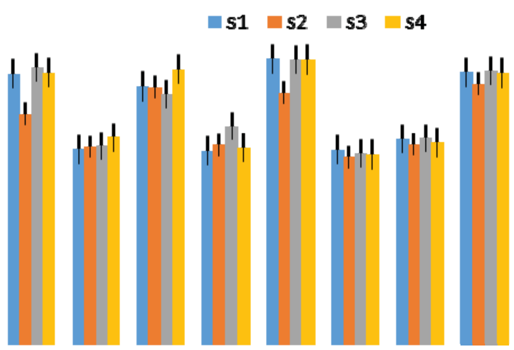

Fig. 5 Seed yield performance of genotypes to drought stresses stages

The lack of interaction between stress and genotypes tested show the uniform effect of stress at various phonological stages on them and can increase the confidence in the results obtained. At flowering stage the stress causes bloom loss, which in turn leads to a reduction in the number of pods. Stress at grain filling stage had minimal impact on the number of pods because stress at this stage, has a very low impact on the number of pods and seeds and will affect mainly on grain weight. Effect of drought stress in different growth stages on yield was significant (P <0.05) (Table I). There was also a significant difference between genotypes in terms of yield (P <0.01), there was also a significant interaction of stress × genotype (P <0.01) (Table II). Since chickpeas is a plant with unlimited growth, therefore, adequate moisture causes plant growth, increased levels of photosynthetic activity and production of more assimilates this in turn, improves the speed and duration of grain filling and finally produced effective transfer of assimilates to grains. The sum of these factors also eventually leads to increased grain yield. On the contrary, drought stress restricts the time needed for more plant growth and optimum transmission of photosynthetic products to grain through induction of precociousness and therefore yield potential decreases [17].

Assessment of the results of the interaction of drought stress and genotypes represents the different behavior of genotypes against the stress in the phonological stage, all genotypes under stress had decreased performance, in the genotypes of MCC392, MCC80 and MCC252, stress emergence in all three stages of growth has created a significant decrease in the performance (Fig. 5) which can be related to their inability to recover after the stress and in other words, their sensitivity is related to stress.

The researchers stated that selection for drought tolerance in chickpea genotypes should be done based on genotypes' yield in stress and non-stress environment, according to this the genotypes with high yield in both stress and non-stress environment are recommended as drought tolerant. Therefore, genotypes MCC283, MCC969 in stress and non-stress conditions had higher performance than other genotypes and they can be considered as part of tolerant genotypes. Although genotypes MCC361, MCC397 also had lower performances under stress conditions and are drought tolerant, but their lower functional potential causes not to be recommended. Chickpeas' yield is a function of the number of pods, number of seeds per pod and weight of 100 seeds. Therefore; effectiveness of each of these components of drought stress can lead to reduced performance that, is although correlated with the percentage of their role in performance changes. Among the yield components the highest positive correlation of yield was with the number of seeds per plant ($r = 0.279$). Based on the results, the last growth stages i.e. flowering and pod of chickpeas are more sensitive to drought stress, however, the incidence of final stress is the most common drought stress, therefore, it seems that in selection of drought tolerant genotypes it is better to consider optimal performance under stress conditions in these stages especially so that, the chance to achieve grow drought tolerant genotypes will be increased.

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