

Research Article

Biochemical and Agronomic Traits of Chickpea Cultivars Response to Drought Stress

Shah T^{1*}, Fareed A² and Nauman M³¹Department of Agronomy, University of Agriculture Peshawar, Pakistan²Institute of Biotechnology and Genetic Engineering University of Agriculture Peshawar, Pakistan³Department of Agricultural Chemistry, University of Agriculture Peshawar, Pakistan***Corresponding author:** Tariq Shah, Department of Agronomy, University of Agriculture Peshawar, Pakistan**Received:** June 14, 2016; **Accepted:** September 01, 2016; **Published:** September 12, 2016**Abstract**

The drought is one of the foremost a biotic stress in agriculture in the world. This study was planned to explore the effect of water stress on the proline content, the chlorophyll content and yield traits in three cultivars of chickpea (KC-98 drought tolerant and KK-2 and sensitive to drought Punjab Noor-2009). An experiment in the field condition with four irrigation schemes has been managed in a randomized complete block design with three repeats. The treatments involved the control (no drought), the water stress forced during the vegetative stage, water stress forced during anthesis phase, and the water stress during the vegetative phase and during anthesis stage. All physiological attributes were affected by the stress of drought. The drought stress appointed during the vegetative growth or anthesis stage drastically reduces the content of chlorophyll a, chlorophyll b and total chlorophyll content. The accretion of proline was superior in the 'KK-2' as compared to "Punjab Noor-2009" at the time under the control and water stress situation. The yields were elevated in the water stress situation than under control condition. In drought conditions the drought responsive variety KC-98" gives the uppermost performance while the variety sensitive to drought, variety 'Punjab Noor-2009" gave the undermost yield. The water stress at anthesis stage diminishes the seed performance more rigorous than that on the vegetative phase.

Keywords: Chickpea; Varieties; Chlorophyll; Proline; Yield**Introduction**

Drought is assuredly one of the most influential environmental stresses that affect the productivity of plants grown in the world [1]. The drought is also an essential performance-inhibiting factor in the chickpea (*Cicer arietinum L.*) production as the main areas of growing of chickpea are in the arid and semi-arid areas and approximately 90% of the global volume of chickpea is grown under rain-fed environments [2] chickpea depicts the mechanisms to overcome this condition. In the chickpea, yield losses can be the result of seasonal drought during the vegetative stage, due to the water stress during reproductive development or by reason of the lethal drought at the end of the crop cycle [3]. The drought stress declines the pace of photosynthesis [4]. The plants grown under condition of drought have a lesser stomatal conductance in order to save water. As a result, the fixing of the CO₂ is diminished and the rate of photosynthesis declined, resulting in reduced assimilates production for growth and the performance of plants. Deviating resistance of stomata to the entry of CO₂ is possibly the foremost factor restraining photosynthesis under drought [5]. Undoubtedly under the mild or moderate water stress (which causes the closing of stomata and reduced leaf internal CO₂ concentration (C_i)) is the main reason of declined rates of leaf photosynthesis [6,7]. Intense drought stress also hinders the photosynthesis of plants by inducing changes in the chlorophyll content, affecting the chlorophyll apparatus and destructing the photosynthetic machinery [8,9]. Documented that leaf chlorophyll content declines as a result of the drought stress. The stress caused by the drought has resulted in a sharp decrease in chlorophyll a, chlorophyll b content, and total chlorophyll content in all varieties

of sunflower seed studied [10]. The diminish in chlorophyll under the drought stress is generally the result of injure to the chloroplasts induced by the active oxygen species [11]. The plants can generally guard themselves against moderate drought by compiling osmolytes. The proline is one of the most familiar appropriate osmolytes in the water stressed plants. For instance, the proline content was amplified under the effect of the drought in pea [12,13]. The accumulation of proline can also be noticed with other stresses, such as a elevated temperature and under the famine [14]. The metabolism of the proline in plants, however, has mostly been calculated in response to osmotic stress [15]. The proline does not hamper with the typical biochemical reactions but permits the plants to endure in conditions of stress [16]. The accretion of proline in the tissues of the plant is also a clear indicator for environmental stress, in particular in the plants under a drought stress [17]. The accumulation of the proline may also be component of the stress influencing adaptive responses [18]. The intention of this study was to provide to an enhanced indulgent of the physiological feedback of the chickpea plants to the water stress. We explore the impact of four types of water stress on the chlorophyll (a, b, a/b) constituents, proline content and yield characters of chickpea cultivars conflicting in the drought tolerance.

Materials and Methods

The study was conducted with three chickpea (*Cicer arietinum L.*) cultivars distinct in the duration of the crop cycle, type (desi or kabuli), behavior of growth and response to the drought: KC-98 (kabuli), KK-2 (kabuli) and Punjab Noor-2009 (desi). The first two are deliberated comparatively tolerant to drought; the last is sensitive to drought. The seeds of these cultivars were collected from the Agriculture Research

Table 1: Metrological data for year 2016.

Month	August	September	October	November	December	
Temperature (°C)	Maximum	20.7	30.9	33.5	38.5	38.6
	Minimum	5.9	14.7	18.9	23.9	27.5
	Average	13.2	22.8	26.5	30.9	32.9
Relative Humidity (%)	37.6	37.9	33.8	30.7	48.2	
Rainfall (mm)	6.9	0	16.2	75.6	41.8	
Average of 10 years Rainfall (mm)	16	18.9	11.8	13.5	57.3	
Sun shine hours (hours)	7	9	11.2	10.5	9.39	
ET0 (mm)	2.6	3.7	5.2	6.3	6	

Source: Weather station at Agriculture Research Station Harichand.

Table 2: Physical and chemical properties of soil prior to sowing.

Parameters	Units	
		0-15 (cm)
Texture	-----	Silt loam
PH	-----	7.7
EC	ds ^m - ¹	0.73
Organic matter	%	0.17
Nitrogen	%	0.052
Available P	ppm	5.1
Extractable K	ppm	138.2
Sand	%	50
Silt	%	22
Clay	%	28
Field capacity	%	25.1
Wilting point	%	7.5
SAR	-----	8

Institute Tarnab Peshawar, Pakistan. The trial was conducted in 2016 in a field of Agriculture Research Station Harichand, Charsadda (34° 8' 43" North, 71° 43' 53" East 282 m above sea level) in Pakistan. The type of soil was the silt loam soil (pH up to a depth of 30 cm was 7.7). The trial was organized in the split-plot arrangement with the three replications. The varieties were taken as sub plot factor and drought treatment as main plot factor. To achieve the drought treatments, plants have been managed to one of the subsequent four irrigation schemes: control; a well irrigated treatment (no water stress), Water stress imposed during the vegetative phase by the withholding of irrigation and the re-watering at and after blossoming, Water stress forced during the anthesis phase by the withholding of irrigation, Water stress forced at both the vegetative stage and anthesis stage in retaining the irrigation. Respective plots were 6 lines (with a row distance of 0.30 m) of a 6 m long. The plant to plant distance was 0.13 m. The plots were irrigated once instantaneously after seeding to guarantee consistent emergence. Subsequently, the plants were watered from the tap in once a week relaying on the treatment at the -2 bar soil water potential. The plots have been kept free of weeds by hand weeding. Surface implementation and adding of 25 kg N ha⁻¹ and 30 kg P ha⁻¹ was done in the framework of the trial. The seeds were inoculated with a fungicide before planting for protection (Tables 1 & 2).

Yield

At the end of the cycle of the crop, the effects of the water stress treatments on the yield of seeds were evaluated. The samples were compiled from an area of 1.0 m² by avoiding the border effects. Also, 5 plants were arbitrarily elected to determine the height of the plant and the number of pods per plant.

Proline content

The evaluations of the proline content were executed twice during the experimental episode, at 40 days (vegetative stage) and 60 days (flowering) after the beginning of the experiment. The proline was squeezed from a sample of 0.5 g fresh leaves in 3% (w/v) aqueous sulphosalicylic acid and approximated with the aid of the ninhydrin reagent according to the [19] method. The absorbance of fraction with toluene sucks from liquid phase has been read at a wavelength of 520 nm. Concentration of the proline was figure out by means of a calibration curve and expressed in μ mol proline G⁻¹ FW.

Chlorophyll contents

The evaluations of the chlorophyll content were conducted twice during the experimental stage, at 40 days (vegetative stage) and 60 days (flowering) after the beginning of the trial. The chlorophyll content was analyzed in 80% extract of the acetone. After the centrifugation (20,000g, 20min) the absorbance was interpret spectrophotometrically at 663 and 645 nm. The total chlorophyll content as well as the concentrations of chlorophyll a and b has been calculated according to the Arnon [20].

Statistical analysis

The data were administered to the Analysis of Variance (ANOVA), and means were correlated using the Duncan's Range test at P = 0.05. All computations have been carried out with the assistance of the SAS software, version 9.1.

Results and Discussion

Chlorophyll

The drought stress forced at the vegetative phase, considerably declined the content of chlorophyll a, chlorophyll b content and total chlorophyll content both at the vegetative phase and the flowering stages, while drought stress established at anthesis also inclined these contents at the time of flowering. The limited water supply during the complete duration of the vegetative state and anthesis had a slight impact on these contents. The absence of effects on the chlorophyll a/b ratio pointed out that the chlorophyll b is not more susceptible

Table 3: Influence of drought stress on chlorophyll contents (mg g⁻¹ fw) at different stages on chickpea varieties.

Treatment	Variety	Chlorophyll A (Mg G ⁻¹ Fw)		Chlorophyll B (Mg G ⁻¹ Fw)		Total Chlorophyll (Mg G ⁻¹ Fw)		Chlorophyll A/B At Flowering
		Vegetative	Flowering	Vegetative	Flowering	Vegetative	Flowering	
Control	KC-98	1.77a	1.52a	0.85a	0.76ab	2.62a	1.99a	2.06abc
	KK-2	1.83a	1.48ab	0.82ab	0.78a	2.54a	1.97ab	1.91bc
	PN-2009	1.77a	1.46ab	0.93a	0.81a	2.70a	1.92ab	1.82c
Drought During Vegetative Phase	KC-98	1.40b	1.13cd	0.56c	0.46d	1.95d	1.58c	2.50ab
	KK-2	1.53b	0.92d	0.72bc	0.46d	2.33b	1.80bc	2.16abc
	PN-2009	1.49b	0.92d	0.62c	0.50cd	2.16c	1.66c	1.86c
Drought During Anthesis Phase	KC-98	-	1.26c	-	0.52cd	-	1.80bc	2.50ab
	KK-2	-	1.23c	-	0.54cd	-	1.65c	2.33abc
	PN-2009	-	1.18c	-	0.55cd	-	1.87ab	2.17abc
Drought During Vegetative and Anthesis Stage	KC-98	-	1.36bc	-	0.54cd	-	1.98ab	2.56a
	KK-2	-	1.37abc	-	0.68ab	-	1.93ab	2.09abc
	PN-2009	-	1.33bc	-	0.63bc	-	1.90ab	2.39abc

The data depicts the mean values of three replications. In the columns means values chased by dissimilar letters are statistically different based the on Duncan's range test at P= 0.05. PN: Punjab Noor

Table 4: Influence of drought stress on proline (μ mol g⁻¹ fw), yield (kg ha⁻¹) number of pods and shoot height (cm) of chickpea cultivars.

Treatment	Variety	Proline (μ Mol G ⁻¹ Fw)		Yield (Kg Ha ⁻¹)	Number of Pods Per Plant	Height of Shoot (Cm)
		Vegetative	Flowering			
Control	KC-98	0.33b	0.68c	2100a	38.7b	18.2b
	KK-2	0.23b	1.27c	1453b	34.2b	22.8a
	PN-2009	0.26b	0.43c	1048c	45.2a	15.5cd
Drought During Vegetative Phase	KC-98	1.65a	8.29ab	1508b	13.5ef	14.1c
	KK-2	1.53a	9.46a	450c	10.2de	15.9bc
	PN-2009	1.63a	8.5ab	708dc	20.2c	11.5e
Drought During Anthesis Stage	KC-98	-	7.37b	1344b	12.1f	17.2b
	KK-2	-	8.30ab	1063c	11.8f	20.2ab
	PN-2009	-	7.31b	628e	18.2cd	15.6c
Drought During Vegetative and Anthesis Stage	KC-98	-	1.01c	813d	7.3g	13.5d
	KK-2	-	1.21c	800d	7.2g	13.9cd
	PN-2009	-	0.60c	358f	10.5fg	11.6c

The data depicts the mean values of three replications. In the columns means values chased by dissimilar letters are statistically different based the on Duncan's range test at P= 0.05. PN: Punjab Noor

to drought than chlorophyll a (Table 3). At the vegetative period variety KK-2 illustrated a higher concentration of chlorophyll a than the other varieties (Table 3). At the stage of flowering, variety Punjab Noor-2009 demonstrated the lowest chlorophyll a content in the four treatments of the stress. The interactions between the variety and the treatment of the water stress were not significant. The differences between cultivars in chlorophyll b and total chlorophyll content at the time of flowering were not significant. The results are in good harmony with [21], who expressed a significant dwindle in chlorophyll a and b induced by water scantiness in six varieties of (*Triticum aestivum*). The diminished or unaffected level of chlorophyll during the drought has been documented in other species, relying on the interval and the intensity of the drought [22]. A decline in total chlorophyll with the drought stress involves a diminution capacity for the harvesting of light. While the manufacturing of reactive oxygen species is generally motivated by an excess absorption of energy in the photosynthetic machinery, this could be refrained by corrupting the absorbing

pigments [23].

Proline

The varietal differences in the proline content or the interactions between cultivar and the treatment of water stress were deficient. The proline content of the leaves, on the other hand, boosted at two stages of growth in all cultivars of chickpea in reply to the drought (Table 3). The boost in proline content due to drought stress was more intense at the stage of flowering that at the vegetative period. The proline content relies on the age of the plant and the leaf age, the position of the leaves or parts of leaf [24]. Under vegetative period, the stress caused by the drought has augmented the proline content approximately ten times, this growing role as an osmotic compatible and regulate osmotic potential which has resulted in a drought stress escaping in the chickpea. It is supposed that the accretion of proline play adjusting roles in the plant stress tolerance [15]. The amassing of proline was recommended as an attribute of selection for tolerance to stress [25,26].

Yield

The yield answer to drought stress of chickpea is specified in (Table 4). The yield of the whole three varieties of chickpeas has been affected by water stress. Stress imposed on plants at the vegetative phase, but not stressed consequently, has given a yield significantly higher than the stress imposed on plants during anthesis, or during the vegetative phase and anthesis phase. The highest performance (under optimum and conditions of water stress) has been achieved from the 'KC-98'. Yield losses in feedback to the stress treatment were: 62% for the "KC-98", 46% for the "KK-2", and 67% for 'Punjab Noor-2009'. Nevertheless, the interactions between the varieties and the treatment of drought were significant. The yield of the seed under the effect of the drought stress at anthesis period illustrated 11% less than the treatment under the drought at the vegetative phase.

Pod number and plant height

The drought had a significant effect on the number of pods and on the height of the plants. The plants were generally taller and had the largest number of pods when they were grown without the stress of drought. The effects of the water stress during the vegetative stage and during anthesis phase on the number of pods were high or less additive, but this was not factual for the effects on the height of shot (Table 4). On average for the whole of the treatments 'Punjab Noor-2009' illustrated the highest number of pods and the shortest plants (Table 4). Although Punjab Noor-2009 had the largest number of pods, it had the lowest performance (Table 4), possibly due to a decline in the percentage of packed pods and the 1000 grain weight. The decline in the yield of grain legumes grown in drought conditions is mainly due to the diminution in the number of pods per plant [27,28].

Conclusion

From this trial it is concluded that, all physiological attributes feedback of drought receptive (KC-98 and KK-2) and sensitive to drought (Punjab Noor-2009) varieties of chick peas to a restricted water supply have publicized similar schemes: decline in chlorophyll a, b, a/b concentrations and the yield were connected with an enhance in the proline. The differences between cultivars were established mainly in water relation traits, which pointed out alteration in the physiology (stomata) or osmotic adjustments. The accumulation of the proline is a common physiological feedback in many plants in reply to drought stress. The photosynthesis is restricted by drought stress due to a stomatal closure and non stomatal (deficiencies of metabolic processes) factors. The drought stress appointed in this study have affected the vegetative growth of both, yield and number of pods of chickpea plants, on the other hand the performance has been the most affected, substantially limiting the number of pods.

References

1. Bohnert HJ, Nelson DE, Jensen RG. Adaptations to environmental stress. *Plant Cell*. 1995; 7: 1099-1111.
2. Kumar J, Abbo S. Genetics of flowering time in chickpea and its bearing on productivity in semiarid environments. *Adv Agron*. 72: 107-138.
3. Serraj R, Krishnamurthy L, Kashiwagi J, Kumar J, Chandra S, Crouch JH. Variation in root traits of chickpea (*Cicer arietinum* L.) grown under terminal drought. *Field Crops Res*. 2004; 88: 115-127.
4. Kawamitsu Y, Driscoll T, Boyer JS. Photosynthesis during desiccation in an Intertidal Alga and a Land Plant. *Plant Cell Physiol*. 2000; 41: 344-353.
5. Boyer JS. Leaf enlargement and metabolic rates in corn, soybean, and sunflower at various leaf water potentials. *Plant Physiol*. 1970; 46: 233-235.
6. Chaves MM. Effects of water deficits on carbon assimilation. *J Exp Bot*. 1991; 42: 1-16.
7. Flexas J, Bota J, Loreto F, Cornic G, Sharkey TD. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biology*. 2004; 6: 269-279.
8. IturbeOrmaetxe I, Escuredo PR, Arrese-Igor C, Becana M. Oxidative damage in pea plants exposed to water deficit or paraquat. *Plant Physiol*. 1998; 116: 173-181.
9. Ommen OE, Donnelly A, Vanhoutvin S, van Oijen M, Manderscheid R. Chlorophyll content of spring wheat flag leaves grown under elevated CO₂ concentrations and other environmental stresses within the ESPACE-wheat project. *Eur J Agron*. 1999; 10: 197-203.
10. Manivannan P, Abdul Jaleel C, Sankar B, Kishore kumar A, Somasundaram R, Lakshmanan GMA, et al. Growth, biochemical modifications and proline metabolism in *Helianthus annuus* L. as induced by drought stress. *Colloids and Surfaces B: Biointerfaces*. 2007; 59: 141-149.
11. Smirnov N. Antioxidant systems and plant response to the environment. In: Smirnov V. *Environment and Plant Metabolism: Flexibility and Acclimation*, BIOS Scientific Publishers, Oxford, UK. 1995.
12. Sanchez FJ, Manzanares M, de Andres EF, Tenorio JL, Ayerbe L. Turgor maintenance, osmotic adjustment and soluble sugar and praline accumulation in 49 pea cultivars in response to water stress. *Field Crops Res*. 1998; 59: 225-235.
13. Alexieva V, Sergiev I, Mapelli S, Karanov E. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell Environ*. 2001; 24: 1337-1344.
14. Sairam RK, Veerabhadra Rao K, Srivastava GC. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Sci*. 2002; 163: 1037-1046.
15. Verbruggen N, Hermans C. Proline accumulation in plants: a review. *Amino Acids*. 2008; 35: 753-759.
16. Stewart CR. Proline accumulation: Biochemical aspects. In: Paleg LG, Aspinall D. *Physiology and Biochemistry of drought resistance in plants*. 1981; 243-251.
17. Routley DG. Proline accumulation in wilted ladino clover leaves. *Crop Sci*. 1966; 6: 358-361.
18. Maggio A, Miyazaki S, Veronese P, Fujita T, Ibeas JI, Dams B, et al. Does proline accumulation play an active role in stress-induced growth reduction? *Plant J*. 2002; 31: 699-712.
19. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. *Plant Soil*. 1973; 39: 205-207.
20. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol*. 1949; 24: 1-15.
21. Nyachiro JM, Briggs KG, Hoddinott J, Johnson-Flanagan AM. Chlorophyll content, chlorophyll fluorescence and water deficit in spring wheat, *Cereal Res Commun*. 2001; 29: 135-142.
22. Kpyoarissis A, Petropoulou Y, Manetas Y. Summer survival of leaves in a soft-leaved shrub (*Phlomis fruticosa* L., Labiatae) under Mediterranean field conditions: Avoidance of photo-inhibitory damage through decreased chlorophyll contents. *Journal of Experimental Botany*. 1995; 46: 1825-1831.
23. Herbinger K, Tausz M, Wonisch A, Soja G, Sorger A, Grill D. Complex interactive effects of drought and ozone stress on the antioxidant defence systems of two wheat cultivars. *Plant Physiol Biochem*. 2002; 40: 691-696.
24. Chiang HH, Dandekar AM. Regulation of proline accumulation in *Arabidopsis thaliana* (L.) Heynh during development and in response to desiccation. *Plant Cell Environ*. 1995; 18: 1280-1290.
25. Yancy PH, Clark ME, Hand SC, Bowlus RD, Somero GN. Living with water stress: evolution of osmolyte systems, *Science*. 1982; 217: 1214-1222.

26. Jaleel CA, Gopi R, Sankar B, Manivannan P, Kishorekumar A, Sridharan R, Panneerselvam R. Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. *South Afr J Bot.* 2007; 73: 190-195.
27. Lopez FB, Johansen C, Chauhan YS. Effect of timing of drought stress on phenology, yield and yield components of a short-duration pigeon pea. *J Agron & Crop Sci.* 1996; 177: 311-320.
28. Pilbeam CJ, Akatse JK, Hebblethwaite PD, Wright CD. Yield production in two contrasting forms of spring-sown faba beans in relation to water supply. *Field Crops Res.* 1992; 29: 73-287.