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Traits of significance for screening of chickpea (*Cicer arietinum* L.) genotypes under terminal drought stress

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is mainly grown in rainfed area and terminal drought stress is one of the major constrain to chickpea production in India. The present study was undertaken to identify the traits of significance that contribute towards terminal drought tolerance in chickpea. The two chickpea genotypes HC-1 (drought sensitive) and RSG 931 (drought tolerant) and F_3 progeny lines of cross HC-1 × RSG 931 were grown in drought microplots and data was recorded for root and physiological traits under terminal drought stress. The results showed wide variation for root and physiological traits in HC-1, RSG 931 and F_3 progeny lines of chickpea. Significant positive correlation was recorded between seed yield and water potential; seed yield and RWC; seed yield and quantum yield of photosystem II, whereas root traits were not positively correlated with seed yield. The results of present study showed that physiological parameters could be reliable tools for screening of terminal drought tolerance in chickpea.
Keywords: Chickpea, physiological traits, root traits, terminal drought stress, seed yield

Chickpea (*Cicer arietinum* L.) is the world's second most important food legume crop grown on low input marginal lands and represents an important component of the subsistence farming (Varshney *et al.,* 2014). Globally, chickpea is cultivated over an area of 14.80 million hectares with an annual production of 14.23 million tons (FAOSTAT 2014). Further, among chickpea growing countries India alone contributes to 70 per cent of the world's total production. Chickpea is one of the most drought tolerant cool-season food legume crops, as it requires only 6-10 inches of rainfall during the completely growing season (Kanouni *et al.,* 2012). However, terminal drought is the one of the most devastating abiotic stress in chickpea because, it is mostly grown on residual moisture from monsoon rains on the Indian subcontinent and the crop matures under progressively declining soil moisture (Turner *et al.,* 2001). It is estimated that, the average air temperature will have risen by 1 to 4°C by 2100 (IPCC, 2013). The temperature rise can reduce the amount of rain and snow, which can enhance the occurrence of drought. Terminal drought can reduce seed yield of chickpea by 58–95 per cent by reducing the number of pods and grain filling duration (Leport *et al.,* 2006).

Deep root system has been proposed as the main target in last two decades for breeding to improve drought avoidance/tolerance in chickpea (Zaman-Allah *et al.,* 2011). It is assumed that deep root system could help in the extraction of available soil water from deeper soil layers. However, Kumar *et al.* (2012) reported that rooting depth and root dry mass of drought tolerant genotype RSG 931 is less as compared to other drought tolerant genotypes still it is performing very well under drought stress conditions. The result of their study showed that some other physiological or biochemical mechanism help plant to cope up with terminal drought stress.

Drought tolerance is a highly complex phenomenon involving many tolerance mechanisms that are inter-related with each other. In recent years,several studies have focused on identification of morpho-physiological traits associated with drought tolerance. There are many traits like water potential(ψ_w), osmotic potential (ψ_p), relative water content (RWC), chlorophyll content, stomatal conductance, root related and yield related traits contributing towards drought stress tolerance (Talebi *et al.,* 2013). Plant water status that includes ψ_w, ψ_p and RWC represents an easy measure of

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water deficit and provides best sensor for drought stress. RWC reduces in response to drought stress in wide variety of plants. The variation in RWC is due to differences in plant ability to absorb water from soil by developing a high ψ_w gradient, increasing root depth and reducing water loss through stomata (Omae *et al.,* 2005). The role of the intact cell membrane remains to be more critical for adaptation of plant in drought stress conditions. It has been reported that drought tolerant genotypes are superior to susceptible ones in maintaining membrane stability and lowering membrane injury under drought stress conditions (Pouresmael *et al.,* 2013). Moreover, quantum yield of photosystem II (F_v/F_m) is also considered as a good indicator of the photosystem II (PS II) activity and its photochemical effectiveness for drought stress tolerance (Govindjee *et al.,* 1981).

The mechanisms of drought stress tolerance depend upon genotypes, growth stages of plants and agro-climatic conditions like severity of water. Hence, identification of suitable drought tolerant traits for screening large numbers of genotypes, in a short time remains a major challenge to the plant breeders (Talebi *et al.,*2013). The present study was undertaken to identify the reliable traits for screening of chickpea genotypes, HC-1, RSG 931 and F_3 progeny lines of cross HC-1 × RSG 931 under terminal drought stress and to correlate them with seed yield.

MATERIALS AND METHODS

The experiment was conducted in drought microplots with rainout shelters at Crop Physiology Field Laboratory, Department of Agronomy, CCS Haryana Agricultural University, Hisar (29010'N latitude, 75048'E longitude, 215 m altitude), Haryana in *rabi* seasons 2012-13. Eighteen F_3 progeny lines of cross HC-1 × RSG 931 along with parental chickpea genotypes (HC 1: drought sensitive and RSG 931: drought tolerant) were planted in specially constructed facilities of concrete microplots (6 m long, 1 m wide and 1.5 m deep connected with iron gates and washing tanks) which were filled with sandy soil and were irrigated up to field capacity. Both the genotypes:HC-1 and RSG 931 were sown in three rows each of 1 m length with inter row spacing of 30 cm and plant spacing of 10 cm under two environments i.e. irrigated (I) and drought (D). In irrigated, two irrigations of 6 cm depth were given, oneat pre flowering and another at pod filling whereas, in drought, only one irrigation of 30 mm was given which equals to long-term average seasonal rainfall. The F_3 progeny lines were sown in one row with the same spacing as in case of chickpea genotypes in drought microplots only. All the recommended agronomic practices were followed for raising the crop.

The soil moisture content was measured at 40, 80 and 120 days after sowing (DAS) at various soil depth levels (0-15, 16-45, 46-75, 76-105 and 106-140 cm) using gravimetric methods (Dirksen, 1999). Roots were taken out from soil at maturity stage after thorough and gentle washing of sand using water jet. Rooting depth and shoot length were measured using meter rod and root/shoot ratio was measured on dry weight basis. Yield of both the parental genotypes, HC-1 and RSG 931 under irrigated and drought stress conditions and F_3 progeny lines under drought conditions was determined at maturity stage. Three plants from parental genotypes as well as from F_3 progeny lines were taken to record the data.

Water potential (ψ_w) of leaf was measured using Pressure Chamber (PMS Instrument Co., Oregon, USA) on a clear sunny day between 10:00 hrs to 12:00 hrs. Osmotic potential(ψ_p) was measured by Vapor Pressure Osmometer (Model 5100-B, Wescor, Logan, USA). Leaf relative water content (RWC) was calculated using Kumar and Elaston (1992) method. Relative stress injury (RSI) was determined by the method of Sullivan and Ross (1979) using conductivity meter. Photochemical efficiency/ quantum yield of photosystem II was determined in intact plants in the field with an OS-30P chlorophyll fluorometer (Opti-Science, Inc., Hudson, NY, USA). Plant water status of third fully expanded leaf (from top) at 50 per cent flowering stage was recorded between 10:00-12:00 hours. The data was analysed to find out the correlation between yield and physiological traits using online OPSTAT statistical software programme (Sheoran *et al.,* 1998).

RESULTS AND DISCUSSION

Soil moisture status

Soil moisture content was measured at 40, 80 and 120 DAS representing vegetative, flowering and maturity phases, respectively, at different layers of soil (Table 1). Soil moisture content was 12.2 per cent up to 15 cm depth at the time of sowing. The soil moisture content was 8.6 per cent and 11.9 per cent at depth range of 76-105 cm and 106-140 cm, respectively at 80 DAS while 8.1 per cent and 10.1 per cent in depth range of 76-105 cm and 106-140 cm, respectively at 120 DAS in drought microplots.The soil surface was almost dry (less than 5.1%) in drought microplots and maximum water was absorbed from the soil depth in the range of 46–140 cm at 80 DAS to maturity (Table 1).

Table 1: Moisture content in different layers of soil profile under irrigated and drought stress conditions at different days sowing

Moisture at		Percent soil moisture at different depths (cm)				
		0-15	16-45	46-75	76-105	106-140
Sowing		12.2	13.4	15.4	16.9	20.4
40 DAS	(I)	6.2	7.1	8.7	13.4	15.9
	(D)	6.0	7.3	8.9	12.7	15.4
80 DAS	(I)	8.7	10.2	13.2	14.8	16.1
	(D)	5.5	6.8	7.9	8.6	11.9
120 DAS	(I)	9.1	10.8	11.6	13.9	15.7
	(D)	5.1	6.5	7.5	8.1	10.1

Where, DAS- days after sowing, I- irrigated, D- drought

Table 2: Root, shoot, physiological traits of leaf and seed yield of parental chickpea genotypes of cross HC-1 × RSG 931 under irrigated and drought stress conditions

Traits	Irrigated		Drought	
	HC-1	RSG 931	HC-1	RSG 931
Rooting depth (cm)	83.8±1.62	77.5±1.15	94.6±1.83	84.6±1.46
Shoot length (cm)	60.4±1.23	58.8±1.27	49.8±1.22	48.4±0.98
Root dry weight (g plant^{-1})	4.32±0.15	3.17±0.12	4.83±0.18	3.9±0.13
Shoot dry weight (g plant^{-1})	7.37±0.25	5.04±0.22	6.51±0.13	4.85±0.13
Root/Shoot ratio	0.58±0.02	0.63±0.01	0.74±0.01	0.80±0.01
Water potential (-MPa)	0.81±0.01	0.78±0.01	1.33±0.02	1.25±0.02
Osmotic potential (-MPa)	1.16±0.02	1.24±0.01	1.37±0.03	1.45±0.03
Relative water content (%)	76.7±0.77	78.7±0.84	59.52±0.37	63.02±0.66
Relative stress injury (%)	17.66±0.49	18.5±0.55	33.3±0.79	26.7±0.89
Quantum yield of photosystem II	0.671±0.008	0.657±0.006	0.467±0.004	0.497±0.005
Seed yield (g plant^{-1})	13.57±0.28	15.20±0.63	7.19±0.23	11.27±0.51

Root traits of HC-1, RSG 931 and F_3 progeny lines of cross HC-1 × RSG 931

Rooting depth and shoot length were less in drought tolerant genotype, RSG 931 than drought sensitive, HC-1 under both irrigated and drought stress conditions (Table 2). Rooting depth increased while shoot length decreased in both chickpea genotypes, HC-1 and RSG 931 under drought stress conditions. The percent increase in rooting depth was more in HC-1 (12.8%) than RSG 931 (9.2%) under drought stress. Similarly, roots dry weight increased while shoots dry weight decreased in both chickpea genotypes under drought stress conditions. Roots dry weight of HC-1 was more than RSG 931. Roots dry weight increased by 23 per cent in drought tolerant RSG 931 while by 11.8 per cent in drought sensitive HC-1 under drought stress conditions. Kumar *et al.* (2012) also reported that rooting depth and root dry mass of RSG 931 is less as compared to other drought tolerant genotypes of chickpea.The results of present study demonstrated that root traits were not contributing towards drought stress tolerance.The conservative use of water in the early vegetative phase might resulted into availability of more water in the upper soil profile during reproductive phase which helped plant to cope up with terminal drought stress (Zaman-Allah *et al.,* 2011). Because of increase in root biomass and decrease shoot biomass, there was more root to shoot ratio under drought stress. The root to shoot ratio of drought tolerant genotype, RSG 931of chickpea was more (0.80) under drought stress condition as compared to irrigated condition (0.63) showing that it can withdraw more water from the soil to maintain water status of the plant under drought stress conditions. Higher root to shoot ratio indicates

Table 3: Range of root, shoot, physiological traits of leaf and seed yield of F_3 progeny lines (P1 to P18) of cross HC-1 × RSG 931 under drought stress conditions

Traits	Range of F_3 progeny lines
Rooting depth (cm)	81.10±1.10 to 98.03±1.41
Shoot length (cm)	42.53±1.11 to 54.87±1.69
Root dry weight (g plant^{-1})	3.47±0.22 to 4.97±0.09
Shoot dry weight (g plant^{-1})	4.42±0.26 to 6.78±0.19
Root/shoot ratio	0.67±0.06 to 0.81±0.04
Water potential (-MPa)	1.35±0.03 to 1.07±0.02
Osmotic potential (-MPa)	1.46±0.03 to 1.31±0.03
Relative water content (%)	56.17±2.55 to 65.07±1.17
Relative stress injury (%)	23.60±0.69 to 33.87±1.22
Quantum yield of photosystem II	0.457±0.004 to 0.533±0.017
Seed yield (g plant^{-1})	6.07±0.34 to 14.09±0.80

partitioning of photosynthatesis more towards root than shoot. Eighteen F_3 progeny lines of cross HC-1 × RSG 931 followed the similar pattern under drought stress conditions.

Physiological traits of HC-1, RSG 931 and F_3 progeny lines of cross HC-1 × RSG 931

Leaf water status: The results of the present study showed that there was decrease in leaf water status of parental chickpea genotypes of cross HC-1 × RSG 931 under drought stress (Table 2). The water potential of leaf (ψ_w) of drought sensitive genotype, HC-1 decreased from -0.81 MPa under irrigated condition to -1.33 MPa under drought stress condition. Whereas, in drought tolerant genotype, RSG 931, ψ_w decreased from -0.78 MPa under irrigated condition to -1.25 MPa under drought stress. Similarly, osmotic potential of leaf (ψ_p) decreased from -1.16 MPa under irrigated conditions to -1.37 MPa under drought stress conditions in HC-1 and from-1.24 MPa under irrigated conditions to -1.45 MPa under drought stress conditions in RSG 931. The decrease in ψ_p of drought tolerant chickpea genotype, RSG 931 was recorded high since it may accumulate higher amount of solutes to cope up with drought stress as compared to HC-1. Ulemale *et al.* (2013) also reported that osmo-regulatory activities helped the plant to cope up with drought stress. RWC of leaf is a widely used to determine the level of internal water status of plants. RWC was recorded relatively low in both chickpea genotypes, HC-1 and RSG 931under drought stress in comparison to non-stress condition (Table 2). The drought tolerant genotype, RSG 931 maintained less decrease in RWC (19.9%) than HC-1 (22.4%). A decrease in the relative water content (RWC) in response to drought stress has been recorded in wide variety of plants including chickpea as reported by Kumar *et al.* (2012) and Talebi *et al.* (2013). High RWC in drought tolerant genotypes may be due to accumulation of osmoprotectants like sugar in plants under drought stress (Gunes *et al.,* 2008).

In F_3 progeny lines, ψ_w, ψ_p and RWC ranged from -1.07 to -1.35 MPa, -1.34 to -1.46 MPa and 56.15 to 65.07 per cent, respectively. Genotypic variation of ψ_w and RWC may be achieved due to differences in the ability to absorb more water from the soil by developing a high water potential gradient from soil to plant and/or the ability to control water loss through stomata (Omae *et al.,* 2005; Siddique *et al.,* 2000).

Relative stress injury and photochemical efficiency of photosystem II: The intact cell membrane is very critical for adaptation of plant in drought stress conditions. It has been reported that drought tolerant genotypes are superior to susceptible ones in maintaining membrane stability under drought stress conditions (Pouresmael *et al.,* 2013). In the present study, relative stress injury (RSI) of HC-1as well as of RSG 931 increased under drought stress conditions. In drought sensitive genotype, HC-1, RSI in irrigated and drought stress conditions was recorded 17.6 per cent and 33.3 per cent, respectively (Table 2). Whereas, in drought tolerant genotype (RSG 931), RSI was recorded 18.5 per cent under irrigated and 26.7 per cent under drought stress conditions (Table 2). Rahbarian *et al.* (2011) also reported that membrane stability significantly decreased in the seedling and early flowering stages under drought stress. In 18 F_3 progeny lines, RSI ranged from 23.6 to 33.8 per cent under drought stress (Table 3). The genotypes/progeny lines that could maintain high RWC and lower RSI had higher tolerance to terminal drought stress.

The quantum yield of photosystem II (F_v/F_m)was significantly reduced under drought stress in both HC-1 and RSG 931 chickpea genotypes. The drought tolerant genotype, RSG 931 maintained higher photosynthetic efficiency than HC-1 in drought stress conditions. In HC-1, $F_v/F_m$was recorded 0.671 under irrigated and 0.467 under drought stress whereas in RSG 931 it was observed to be 0.657 under irrigated and 0.497 under drought stress (Table 2). The $F_v/F_m$was recorded in the range of 0.457 to 0.533 in F_3 progeny lines (Table 3). Several researchers have reported that drought stress caused degradation of PS II oxygen-evolving complex and the PS II reaction centers (Lu and Zhang, 1998; Maxwell and Johnson,2000; Murata *et al.,*

Table 4: Correlation matrix of physiological traits with seed yield of F_3 progeny lines of cross HC-1 × RSG 931

	SY	WP	OP	RWC	RSI	F_v/F_m
SY	1					
WP	0.644^{**}	1				
OP	0.325	0.150	1			
RWC	0.617^{**}	0.642^{**}	0.038	1		
RSI	-0.518^{*}	-0.606^{**}	-0.232	-0.335	1	
F_v/F_m	0.518^{*}	0.325	0.046	0.549^{*}	-0.560^{*}	1

Where, SY- seed yield per plant, WP- water potential of leaves, OP- osmotic potential of leaves, RWC- relative water content, RSI- relative stress injury, F_v/F_m - quantum yield of photosystem II,

*- significant at 0.05, **-significant at 0.01

2007). RWC was found to be positively correlated with F_v/F_m in the present study (Table 4). Generally, it has been observed that genotypes with higher RWC have a higher photosynthetic ability under drought stress (Siddique *et al.,* 2000).

Correlation between physiological traits and seed yield

Drought stress resulted in reduced seed yield of both the parental genotypes as well as of 18 F_3 progeny lines of HC-1 × RSG 931. This may be due to reduction in reproductive phase and pod abortion or reduced pod filling duration. Leport *et al.* (2006) had also observed a significant yield loss due to pod abortion. The yield of both HC-1 and RSG 931was decreased in drought stress condition but decrease in yield of RSG 931 (25.8%) was less as compared to HC-1 (47%). Water potential, RWC and $F_v/F_m$of 18 F_3 progeny lines of cross HC-1 × RSG 931 had a significant positive correlation with seed yield whereas, RSI showed negative correlation with seed yield (Table 4).The results of correlations revealed that water potential had the largest effect on seed yield. Summy *et al.* (2015) also reported positive correlation of ψ_w, RWC, and F_v/F_m with seed yield.

The implications of the results revealed that the physiological traits had a direct effect on seed yield while deep rooting and higher root biomass had no positive effect on seed yield. Hence, physiological parameters like ψ_w, RWC, $F_v/F_m$and RSI could be used for screening of chickpea during early reproductive stage and could be exploited in crop improvement programmes for development of drought tolerant genotypes.

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