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Root length and leaf cuticular wax: The traits associated with drought avoidance in sunflower hybrids

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Abstract

It is well known that the root traits and accumulation of leaf epicuticular wax would bring the drought avoidance in sunflower both in terms of absorption of water and reduced transpiration respectively. Studies on root system using containers with sufficient volume for potential root growth are not available among popular hybrids. Hence, present experiment was formulated to study the physiological and biochemical basis in imparting drought resistance in popular sunflower hybrids. Results revealed that, the shoot traits were least affected as compared to root traits signifying the relevance of root traits on drought avoidance mechanism in sunflower. Improvement in root traits under stress conditions to cope up with drought conditions. Among the traits studied, the dry matter production was highly related to root length and leaf cuticular wax content by correlation analysis, multiple linear regression and step wise regression analysis. Hence, the root length and leaf wax content are the most significant traits which can be considered in breeding programmes aid at drought resistance.

Keywords: Biomass, growth, proline, stress

Introduction

Sunflower (*Helianthus annuus* L.) is an important oilseed crop after soybean and groundnut. It has superior oil quality with high oleic acid (25 to 30%) and linoleic acid (64 %) contents (Rodriguez *et al.*, 2002) [40]. In India, the area under cultivation has been in decreasing order and it is cultivated in an area of 0.28 million hectares with a production of 0.17 million metric tonnes and productivity of 0.61 metric tonnes per ha⁻¹ (USDA, 2020) [54]. The major environmental limitation that limits the overall production is inadequate soil moisture to meet the transpiration demands due to drought situations during monsoon seasons. Sunflower is mainly cultivated as rainfed crop and thus exposed to drought stress conditions and reduces productivity. Several attempts have been made to study the drought tolerance in sunflower. Most of the studies are pertains to above ground parameters such as physiology of leaf and biochemical composition of leaves, but not the root traits. A few studies available on root studies were mostly confined to early stages (seedling stage and laboratory conditions using small containers). The root grows deep up to the flowering stage; hence, we attempted to study the root characteristics using big containers to support sufficiently the root growth. Further, evaluation of popular hybrids for their physiological and biochemical performance is scanty. Hence, studies in large containers would aid in selection of traits responsible for drought resistance.

Sunflower is highly responsive to irrigation (Pejic *et al.*, 2009; Sezen *et al.*, 2019) [32, 46] and moisture stress decreases the seed yield (Silva *et al.*, 2015; Sezen *et al.*, 2019) [50, 45]. Furthermore, in the changing climate scenario, drought stress is likely to be continued as major abiotic limitation. Drought stress leads to considerable decrease in seed yield, yield components and seed oil content of sunflower (Alahdadi *et al.*, 2011) [2]. Productivity of crop is the product of biomass and harvest index (Nanja Reddy *et al.*, 1994; Nanja Reddy *et al.*, 2003, Alahdadi *et al.*, 2011; Praveen *et al.*, 2018; Pious Secondo and Nanja Reddy, 2019) [26, 27, 2, 35, 33]. Of these, increase in dry matter production keeping the harvest index constant could enhance the productivity. Therefore, the associated parameters with dry matter production could be important for exploitation of genetic resources and inclusion in breeding programmes.

Of the several morpho-physiological traits that influence the biomass and seed yield under stress conditions, root traits are important for uptake of water, and to avoid the drought. The crop growth and biomass production of a given crop primarily depend on root system which helps in efficient uptake of water and minerals. Further, a better root system interacts with soil microbes, increases the nutrient release and uptake, provides anchorage and drought tolerance (Lynch and Brown, 2012) [21]. Response of roots to the drought has been reported earlier in sunflower hybrids and inbred lines (Angadi and Entz, 2002; Rauf *et al.*, 2009) [4, 38]. The higher root growth is linked to better drought tolerance but screening genotypes for root characters is laborious (O'Toole and Bland 1987) [29]. However, efforts have been made using pot culture studies but most of the studies are restricted to early growth phases (Rauf *et al.*, 2009) [38]. Hence there is a need for root studies at later stages of the crop especially using large containers to support potential growth of roots.

In addition to root traits, the above ground leaf characteristics are important in imparting drought tolerance. It could be closure of stomata to maintain the leaf water status minimal to run the metabolic activities, which include stomatal modifications, biosynthesis and accumulation of osmolytes like proline (Sayed *et al.* 2012) [43], hormones and secondary metabolites (Seki *et al.*, 2007; Mouillon *et al.*, 2008; Seo, 2009) [44, 25, 45]. Drought stress firstly decrease the leaf water potential, cell enlargement, and cell growth with closure of stomata due to loss of turgor (Kaya *et al.*, 2009) [19]. Thus reduces the leaf area development, photosynthesis and biomass production (Cechin *et al.*, 2015) [9]. Another important trait is leaf cuticular wax, which play an important role in plant resistance to both abiotic and biotic stresses, such as those caused by fungal pathogens, phytophagous insects, freezing temperatures, and drought by reducing the non-stomatal transpiration (Kerstiens, 1996; Buda *et al.*, 2013) [20, 7]. Therefore, we made an attempt to examine the effect of moderate and severe moisture stress on various morpho-physiological and biochemical traits, the relationship between the traits and contribution of each trait to the biomass production in sunflower hybrids.

Materials and method

Experiments were conducted at the Department of Crop Physiology, University of Agricultural Sciences, GKVK, Bangalore during *khari*f 2017. In the present investigation, 17 popular sunflower hybrids were evaluated for their morpho-physiological and biochemical responses to different moisture stress conditions in two replications. The seeds were sown in pots directly on 08th May 2017 and thinning was carried on 10th day after sowing (DAS) to maintain single plant/pot. The pot size was 38 cm height x 28 cm diameter with a capacity to hold 30kg soil. Three levels of stress were created as control (100 % FC), moderate (70 % FC) and severe (40 % FC) considering the field capacity of soil (22%). Before sowing, pots were filled with 30kg of soil and then sowing was taken up and one seedling per pot was maintained. The pots were added with recommended dosage (60:90:60) Kg/ha of fertilizers based on soil volume in the pot. All the pots were irrigated to maintain 100% FC up to 25th DAS, from 26th DAS to 55 DAS, 70 and 40 % FC was maintained by gravimetric approach (Udayakumar *et al.*, 1998) [53]. The individual container was weighed daily using a mobile electronic load cell balance of 60 kg capacity in view of maintaining respective moisture levels. The load cell balance was fixed on a mobile gantry system with a provision for movement along

the rails horizontally to access every pot. The containers were placed in mobile ROS to protect from any external moisture entry (rain interruption). During the end period of stress, physio-morphological and biochemical parameters were recorded followed by the pots were irrigated to saturation level for easy uplift of plant from the pot so as to measure root traits. The morpho-physiological traits, plant height (cm), number of leaves per plant, total biomass (g/plant), SPAD Chlorophyll Meter readings (SCMR), relative water content (%), rate of water loss (%), root length (cm), root volume (cc) and root dry weight (g) were measured during the end of stress period. The biochemical parameters, proline, leaf surface wax and melonaldehyde (MDA) content were measured during the same period.

Statistical analysis

The data collected were analysed for ANOVA using OPSTAT (Sheoran *et al.*, 1998) [48]. Correlations between different parameters were analysed in EXCEL programme. Multiple linear regression (MLR) analysis was carried in SPSS programme.

Results and Discussion

Drought is one of the abiotic stress that limits the crop productivity through decreased physiological processes (Erdem *et al.*, 2006; Ghaffari *et al.*, 2012) [11, 13]. The morpho-physiological traits decreased at a greater rate due to severe stress as compared to moderate stress (Table 1). Among all the traits, root dry weight was most affected and SPAD chlorophyll meter reading was least affected. However, a few biochemical parameters, proline, melonaldehyde and wax contents were increased with moisture stress (Table 1).

Days to 50 % flowering was decreased marginally due to stresses infer that moisture stress have no much influence on flowering. However, Buriro *et al.* (2015) [8] reported, an early flowering with a less number of irrigations. Early flowering reduces the thalamus size and thus seed yield. Therefore, it can be suggested that the time taken for flowering will be affected by stress depending upon the duration and magnitude of stress. The plant height was decreased by 16.1% and 33.2% due to moderate and severe stress respectively. Similarly, Mobasser and Tavassoli (2013) [24] have shown decreased plant height, biomass accumulation and the seed yield due to less number of irrigations. Decreased plant height could be due to synthesis of ABA due to decreased water potential caused by drought stress (Sharp, 2002; Nezami *et al.*, 2008) [47, 28], and ABA signalling cause closure of stomata, reduced nutrient absorption and plant height (Tiaz and Zeiger, 2009) [52]. Leaf area is an important trait in determining the seed yield under optimal conditions / protective irrigations (Nanja Reddy *et al.*, 2003) [27]. However, under stress conditions the leaf area would be decreased as an adaptive mechanism to reduce the water loss through transpiration (Silva *et al.*, 2017) [49]. The number of leaves per plant which contribute to leaf area was reduced by 5.8% and 13.1% respectively in moderate and severe stress (Table 1; Freitas *et al.*, 2012) [12]. Reduction in leaf number could be due to reduction in cell division and cell enlargement especially in severe stress (Robertson *et al.*, 1990; Sacks *et al.*, 1997) [39, 41]. The SPAD is an index of chlorophyll content, which determines the photosynthetic rate and assimilates production for plant growth and productivity. The SPAD has relevance on leaf area, plant height, shoot dry matter production and grain yield under stress condition (Ghaffari *et al.*, 2012) [13]. The SPAD value may not change differ with 60 % FC, however severe

stress (30 % FC) decreases the SPAD (Nezami *et al.*, 2008) [28]. In the present study, the SPAD value was decreased by 4.7 % and 9.1 % respectively at 70 % and 40 % FC (Table 1), the decrease in SPAD values under stress condition could be due to photo-oxidation and degradation of chlorophyll (Smirnov, 1995) [51]. The SPAD value has positive relation to growth and productivity of sunflower (Ghaffari *et al.*, 2012) [13] hence, hybrids with lesser reduction in SPAD under stress condition could be better.

The crop growth and productivity of a given crop primarily depend on root system which helps in efficient uptake of water and minerals, interaction with soil microbes, nutrient release, provides anchorage and drought tolerance (Lynch and Brown, 2012) [21]. The root length was increased by 19.1% and 74.8% with moderate and severe stress respectively as compared to non-stressed plants. In contrast, their respective root dry weight and root volume were decreased under stress condition (Table 1). This suggests that under stress condition, the root becomes thin and longer to harness water and minerals from deeper layers. Hence, it would be appropriate to look for variability in root length under stress condition. Similarly, sunflower hybrids with deeper root did not possess higher root volume and root dry weight under stress condition (Martin *et al.*, 1983; Ahmad *et al.*, 2009) [1]. Drought stress known to restrict the lateral root growth (Chen *et al.*, 2005) [10] due to diversion of photosynthates for root length for survival rather than producing more number of roots. In other words, Santhosh *et al.*, (2017) [42] reported a decrease in root length under low concentration of PEG stress (-0.2 MPa) suggesting that sufficiently large pot culture (near to the field condition) shall be used for screening genotypes for drought tolerance in sunflower.

Significant reduction in RWC was observed in moderate (10.9 %) and severe stress (18.4 %) condition, which could be due to decrease in turgor pressure in plant cells and reduced water potential in root (Baldini *et al.*, 2000; Rauf and Sadaqat, 2008; Ghobadi *et al.*, 2013) [6, 37, 14]. Rate of non-stomatal water loss was less in severe (10.9%) and moderate (13.4%) stress conditions compared to control (15.5%) due to increased wax accumulation with stress (Table 1). The proline, an osmolyte is known to accumulate under stress conditions (Alia *et al.*, 2001) [3] was increased by 127.8 % at moderate stress and 287.7 % at severe stress conditions (Table 1), which might be due to increased lipid peroxidation and or by production of free radicals (Ashraf and Foolad, 2007) [5]. In addition, MDA content was increased by 187.8 % and 74.6 % under moderate and severe stress respectively (Table 1). Change in MDA content could be due to increased peroxidation lipid membrane by reactive oxygen species (ROS) such as O₂, H₂O₂ and OH radicals (Mittler, 2002) [23]. Significant increase in leaf cuticular wax was observed under severe stress (70.3 %) followed by moderate stress (36.5 %) which in turn results in decreased water loss to avoid leaf transpiration (Matin *et al.*, 1989; Jongdee *et al.*, 2002; Porcel and Ruiz-Lozano, 2004) [22, 17, 34]. The reduction in various morpho-physiological traits lead to a reduction of dry matter production by 31.9 % and 56.8 % respectively under moderate and severe stress conditions (Table 1; Silva *et al.*, 2017) [49] which determines the crop productivity.

Understanding the relationship between various physiological and biochemical traits those impart the drought tolerance are useful in crop improvement programmes. Correlation coefficients show the interrelationship between the characters quantitatively. Among the root traits, the root length had positive relationship with TDM under well watered condition

($r=0.55^*$) but the relationship was weak under moderate or severe stress conditions (Table 2). Under severe stress conditions, the root length showed significant positive relationship with wax content ($r=0.73^{**}$) and negative relationship with rate of non stomatal water loss (rate of water loss in detached leaves) ($r=-0.64^{**}$) and MDA ($r=-0.50^{**}$), suggests that root length accompanied by wax accumulation on leaf are better traits for rainfed situations. Hence survival under severe drought stress is important rather than the dry matter accumulation. The root dry weight had no significant relationship with any of the traits both in control and stress conditions (Table 2). However, in control condition, the RDW was negatively related to wax content ($r= -0.46^{NS}$), wherein root mass is important for absorption of water and nutrients and wax accumulation is not required under adequate moisture conditions. Surprisingly, the root volume under control condition is positively related to wax accumulation, probably more the wax, the reduced water loss, which might be accumulated in root and hence higher root volume but not the root dry weight. The relationship is also true under severe stress condition ($r= -0.41^{NS}$), this reiterates the importance of wax content for making availability of water to the roots for proper root metabolic activities for survival under severe stress.

The rate of water loss (RWL) not related to any of the physiological traits and biochemical traits under well watered conditions, where water is not a limitation (Table 2). However under moderate drought stress condition, RWL was negatively and significantly related with proline content ($r= -0.72^{**}$) and wax content ($r = -0.53^*$). While under severe stress condition, RWL was related negatively with proline ($r= -0.42^{NS}$), wax content ($r= -0.85^{**}$) and TDM ($r = -0.58^*$) and; positively with MDA ($r= 0.56^*$). These results infer that, under stress conditions, these traits have a significant role in imparting drought tolerance. The leaf relative water content, found to have a positive influence in drought tolerance to maintain normal metabolic processes (Qamar *et al.*, 2018) [36], in the present experiment under severe stress condition, the RWC was positively and significantly related with proline ($r= 0.72^{**}$ in Table 2; Qamar, 2018) [36] and TDM ($r= 0.72^{**}$). Signifies that under stress condition, proline gets accumulated, which lower the water potential of cells, thus water entry from soil to maintain higher leaf water potential, metabolic activities and dry matter production.

The osmolyte accumulation is one of the strategies of survive under stress conditions (Rauf and Sadaqat 2007). The proline content was positively and significantly related to wax content both in moderate ($r= 0.59^*$) and severe stress ($r= 0.52^*$). The proline also had positive significant relationship with TDM ($r = 0.54^*$; Rauf and Sadaqat 2007) under severe stress condition. The melanoaldehyde content refers to peroxidation of membrane, higher the melonaldehyde more the lipid peroxidation, damage to cell membrane, thus have negative relationship with proline content ($r= -0.64^{**}$) and biomass production ($r = -0.49^*$) under severe stress condition. The wax content towards biomass production was positive only in severe stress condition ($r = 0.51^*$), implies that wax content is more important in severe stress conditions rather than mild stress for productivity of sunflower.

These results suggest that under stress condition accumulation of proline lead to more accumulation of wax and total dry matter. Proline accumulation leads to maintenance of higher RWC and leaf cuticular wax. In contrast, it decreases the MDA content and non-stomatal water loss and thus the dry matter production depends on these traits. It appears both

proline and wax accumulation goes hand in hand, to reduce the water loss by wax and increase the RWC by proline, thus survival of plant under stress condition.

To identify contribution of these physiological or biochemical traits towards biomass production, multiple linear regression was followed. It is clear from the regression coefficients, that leaf wax content has highest positive contribution towards biomass (12.09), followed by rate of water loss (1.98) and relative water content (1.75) and; the MDA (-1.83) and proline contents (-1.12) were negatively influenced the biomass production (Table 3). Thus, reflects the importance of these variables in maintaining the TDM through avoidance mechanism under severe stress condition by reducing water loss due to reduced transpiration rates accomplished by wax

accumulation (Matin *et al.*, 1989; Jongdee *et al.*, 2002; Porcel and Ruiz-Lozano, 2004; Goodwin and Jenks, 2005; O'Toole *et al.*, 1979; Jordan *et al.*, 1983; O'Toole and Cruz 1983; Jefferson *et al.*, 1989; Zhang *et al.* 2005) [22, 17, 34, 15, 31, 18, 30, 16, 55]. In addition, contribution of these traits towards dry matter production was confirmed through stepwise regression. This analysis showed the most contributing significant factors were the root length and leaf epicuticular wax.

From this study it can be concluded that, drought stress at grand growth stage decrease the plant biomass production. Hence, in development of sunflower hybrids for drought stress conditions / rainfed conditions, parents selected for high leaf wax and root length are useful.

Table 1: Effect of moisture stress on morphological and physiological traits in sunflower hybrids

Parameters	Control 100 % FC	Moderate stress 70% FC	% reduction over control	Severe stress 40 % FC	% reduction over control
Days to 50% flowering	56.9	55.8	2.0	55.0	3.4
Plant height (cm)	114.7	96.3	16.1	76.6	33.2
No. of leaves per plant	20.4	19.2	5.8	17.7	13.1
SCMR	39.0	37.2	4.7	35.5	9.1
Root length (cm)	24.1	28.7	-19.0	42.1	-74.8
Root dry weight (g/plant)	16.1	9.0	44.1	5.1	68.3
Root volume (cc)	51.5	30.1	41.4	20.8	59.5
Rate of water loss (%)	15.5	13.4	13.6	10.9	30.2
Relative water content (%)	71.6	63.8	10.9	58.4	18.4
Proline content ($\mu\text{g g}^{-1}$ FW)	3.2	7.3	-127.8	12.5	-287.7
Melondealdehyde ($\mu\text{g g}^{-1}$ FW)	3.6	6.3	-74.6	10.4	-187.8
Wax content ($\mu\text{g cm}^2$)	4.9	6.7	-36.5	8.4	-70.3
Total dry matter (g/plant)	71.3	48.6	31.9	30.8	56.8

Note: 1. Stress levels were maintained gravimetrically on daily basis

2. Observations were made from 50 to 55 DAS (ending stage of stress period)

Table 2: Pearson correlations between morpho-physiological traits in sunflower hybrids under (a) control, (b) moderate stress and (c) severe stress conditions

100%	RL	RDW	RV	RWL	RWC	Proline	MDA	Wax
RDW	0.34							
RV	0.31	0.34						
RWL	-0.24	-0.29	-0.48					
RWC	-0.15	0.19	-0.33	-0.08				
Proline	0.25	-0.19	0.12	0.15	-0.50*			
MDA	-0.11	-0.21	0.05	0.23	-0.24	0.43		
Wax	-0.03	-0.46	0.49*	-0.03	-0.22	0.10	0.26	
TDM	0.55*	0.28	0.38	0.00	-0.24	0.29	0.47	-0.05
70%	RL	RDW	RV	RWL	RWC	Proline	MDA	Wax
RDW	0.51*							
RV	0.40	0.48						
RWL	-0.37	-0.31	0.01					
RWC	0.24	-0.27	-0.32	-0.14				
Proline	0.35	-0.04	-0.11	-0.72**	0.14			
MDA	0.27	0.07	-0.21	0.02	0.13	0.01		
Wax	0.15	0.00	-0.24	-0.53*	-0.08	0.59*	0.00	
TDM	0.28	0.42	-0.06	-0.25	-0.05	0.09	0.27	0.18
40%	RL	RDW	RV	RWL	RWC	Proline	MDA	Wax
RDW	-0.10							
RV	0.28	0.12						
RWL	-0.64**	-0.20	-0.40					
RWC	0.25	0.20	-0.05	-0.45				
Proline	0.36	-0.06	-0.03	-0.42	0.72**			
MDA	-0.50*	0.14	-0.08	0.56*	-0.62**	-0.64**		
Wax	0.73**	0.03	0.41	-0.85**	0.36	0.54*	-0.38	
TDM	0.31	0.18	-0.10	-0.58*	0.72**	0.54*	-0.49*	0.51*

Note: (1)* and ** denotes significance at 0.05 and 0.01 level respectively

(2) RL: Root length, RDW: Root dry weight, RV: Root volume, RWL: Rate of water loss, RWC: Relative water content and MDA: Melondealdehyde

Table 3: Relative contribution of physiological traits for dry matter production in sunflower across the stress levels

Traits	Coefficients	Standard Error	t Stat	P-value
Intercept	-161.41	50.16	-3.22	0.01
Rate of water loss	1.98	0.91	2.17	0.05
Relative water content	1.75	0.48	3.65	0.00
Proline content	-1.12	1.19	-0.94	0.37
Melonaldehyde	-1.83	0.92	-1.98	0.07
Wax content	12.09	4.36	2.77	0.02

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