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CHARACTER ASSOCIATION AND PATH ANALYSIS OF OSMOTIC ADJUSTMENT, GROWTH AND PHYSIOLOGICAL TRAITS IN WHEAT

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ABSTRACT

Osmotic adjustment is an important physiological mechanism to sustain plant growth and productivity under drought stress. Plants maintain their turgor potential by accumulating osmolytes which decrease the osmotic potential. Present experiment on traits association and path analysis was conducted during 2012-2013, under two environments (control and drought stress). This study provided insight into correlation among six physiological and four growth traits and their cause and effects were determined. The traits proline, glycine betaine and sugar were observed to have highly significant positive correlations with osmotic adjustment, both at genotypic and phenotypic levels under drought stress. Path coefficient analysis study under two diverse (control and drought stress) environmental conditions revealed that the traits viz. shoot length, root length, fresh root weight, sugar and glycine betaine demonstrated the differential behavior for osmotic adjustment. Positive direct effects observed by shoot length (1.1053), root length (1.8386), fresh root weight (0.4742), sugar (3.3994) and glycine betaine (1.1224) on osmotic adjustment under drought stress. The direct effects convinced that the selection on the basis of root length, sugar and glycine betaine traits was considered of great importance for cultivar development with considerable drought tolerance at early growth stage.

Keywords: Drought, Osmotic adjustment, Correlation, Path analysis, Wheat.

INTRODUCTION

Drought is one of the major abiotic stresses, which drastically threats wheat productivity and yield worldwide (Shiri *et al*., 2010). Drought stress is the characteristics of arid and semi-arid environments worldwide where limited moisture poses a devastating effect to wheat production, while 35% of the total area of developing and poor countries is semi-arid in nature. In Pakistan approximately one third of the total cultivated land lies in the rainfed environment (Alam, 2000). Water deficit affects the crop growth and productivity through modifying the morphological, anatomical, physiological and biochemical activities in plants.

Selection and breeding strategies for production enhancement under water deficit environment has been key aspect of any breeding program. Recent studies implicit that physiological traits based selection has potential to improve genetic yield gain in wheat (Reynolds, 2002). The physiological basis for drought tolerance is still poorly implicit. Breeding for improved crop performance under drought condition has largely relied on direct morphological phenotyping (Fleury and Jafferies, 2010).

Plant adopted different responses when they encounter water deficit environment. Osmotic adjustment is among crucial physiological response against drought stress which enables plants to sustain their growth in decreased water supply. Osmotic adjustment is complex physiological process which results from the interaction of different growth and physiological traits. As osmotic adjustment maintained turgor potential at lower water situation by accumulating osmolytes (viz. proline and sugar); dehydration responsive functions (i.e. excised leaf water loss and deeper rooting system).

Inter-relationship among these factors is quantified through correlation analysis. Phenotypic and genotypic correlations within varieties indicated the degree to which various physio-morphic characters are associated with osmotic adjustment. The prevalence of correlation between different physiological and growth traits with osmotic adjustment under drought stress is of great importance for many plant breeders. These factors also influenced the osmotic adjustment capacity both directly and indirectly. Path analysis described whether the influence of these factors directly affect osmotic adjustment or taken some other indirect way for ultimate effect. Development of drought tolerant genotypes thus necessitates a comprehensive study of correlated response of different physiological traits.

The objectives of the present study were (i) to investigation of the feasibility of employing secondary selection traits to identify drought tolerant genotypes, (ii) to collect information on the association of various physiological traits and (iii) to evaluate the best traits associated with osmotic adjustment under drought stress.

MATERIALS AND METHODS

Plant Materials and Growing Conditions: The research material was comprised of thirty wheat genotypes of Pakistan. These genotypes were collected from different institutes including PMAS- Arid Agriculture University, Rawalpindi; Barani Agricultural Research Institute (BARI), Chakwal and Wheat Research Institute (WRI), Faisalabad. Experiment was conducted in glass-house of PMAS- Arid Agriculture University Rawalpindi (at the latitude of 33.40° N and 44.30° E), during 2012-13. Thirty wheat genotypes were sown in a triplicated Complete Randomized Design (CRD) in polythene bags (12×6 inch size). These genotypes were evaluated at seedling stage for physiological and growth traits under two diverse environmental conditions. Polythene bags were filled by soil mixture having field soil, sand and farm yard manure in 3:1/2:1 proportions and maintained in glasshouse conditions viz. 20±2°C temperature, under natural day/light. Eight seeds were grown in each bag and after germination seedlings were thinned to four.

Drought Stress Imposition: This experiment was conducted in two sets, with the aim to evaluate the impact of stress on studied traits in relation to normal growing plants at seedling stage. First set of the experiment, referred to as control (well-watered conditions), plants were watered normally whenever required and second set, referred to as drought stressed where watering interrupted for 15 days. Drought stress was imposed by withholding water as done by Gusmao *et al*. (2012). When plants reached at tillering stage, watering was interrupted for 15 days for stressed pots as reported by Bajji *et al*., (2001) and control pots were watered normally. After fifteen days of drought stress the data were recorded.

Measurement of Physiological Traits: Proline was assayed according to the ninhydrin method (Bates *et al.*, 1973). Glycine betaine contents were measured according to the method proposed by Grieve and Grattan (1983). Total sugar contents were analyzed according to the anthrone method (Yemm and Willis, 1954).

Excise leaf water retention was recorded according to Farshadfar *et al*. (2002). Fresh leaves were detached and immediately, recorded the fresh weight (FW), left for 4h, followed by wilting at 25°C and reweight (W4h). ELWR was calculated using the following formula: ELWR (%) = [1 - ((FW - W4h)/FW))] × 100

Cell membrane stability (CMS) estimated according to in-vivo procedure to evaluate the intensity of field stress (Tripathy *et al*., 2000). CMS was calculated using the following formula: CMS%= {(1-(T1/T2)) / (1-(C1/C2))} x100. Where, T_1= Stress sample conductance before autoclaving; T_2= Stress sample conductance after autoclaving; C_1= control sample conductance before autoclaving and C_2= control sample conductance after autoclaving.

Osmotic adjustment will be measured by the rehydration method of Blum (1989). Leaves were placed at -20^{0}C in deep freezer for at least three days of both treatments. After three days, samples were thawed and extracted the cell sap. The solute potential of the leaf sap were measured by osmometer. Osmotic adjustment was calculated by using the formula: OA = OP non-stressed – OP stressed

Measurement of Growth Traits: After the physiological measurements, roots and shoot washing were carried out. Immediately after washing following parameters were recorded. Shoot length (SL), root length (RL) of all seedlings was measured by using scale. Fresh soot weight (FSW) and fresh root weight (FRW) was recorded after removing surface water by blotting.

Statistical Analysis: Analysis of variance was carried out with help of M-STATC software. Genotypic and phenotypic correlation coefficients were computed among the traits according to the method given by Known and Torrie (1964). Path analysis of above listed traits on osmotic adjustment was also conducted according to Dewey and Lu (1959).

RESULTS AND DISCUSSION

In this study, analysis of variance revealed highly significant differences for all characters including growth traits like shoot length, root length, fresh shoot weight, fresh root weight and physiological traits i.e. excised leaf water retention, proline, sugar, glycine betaine, cell membrane stability and osmotic adjustment (Table 1). This indicated the sustainable genetic diversity exist in the studied material for these traits which can be exploited in future breeding program. Treatment mean square also revealed highly significant results for all studied traits (Table 1). These results depicted that stressed and non-stressed treatments showed positive effects in all traits. It also suggested that variation exist among the genotypes over the environments. Mean square values of treatments into genotypes interaction showed significant results for glycine betain (GB), highly significant results for all studied traits (Table 1). Interaction among treatments into genotypes (T×G) revealed that treatments showed significant effect on genotypes among all the studied traits. Selection on the basis of these traits was effective for breeding program. Significant results reflected that genotypes' tolerance level against drought stress varied.

Correlation Coefficient Analysis: Correlation coefficient is a measure of a linear relationship between characters. It measures the mutual relationship between two variables but does not let to know the cause and effect relationship of traits adding directly or indirectly towards economic yield (Shivanna *et al*., 2007). The study of associations with quantitative traits is essential for assessing the possibility of joint selection of two or more characters and thus, for evaluating the effect of selection for secondary traits on genetic gain for the primary trait under consideration. In the present study, reciprocal relationship between plant growth and physiological traits in Table 2 and Table 3 demonstrated that the phenotypic correlation coefficients were lower than the corresponding genotypic correlation coefficients. Such correlations revealed the strong inherent relationship between characters. Environmental factors modified the phenotypic expression of these traits by decreasing the phenotypic coefficient values. Such suppressing behavior of environmental factors in reducing the phenotypic correlation coefficients was also reported by Majumder *et al*. (2008) in spring wheat (*Triticum aestivum* L.).

Table 1. Mean square of physiological and growth in 30 wheat genotypes under normal and drought stress conditions.

Variables	Genotypes	Treatments	T×G
Shoot Length	10.584**	201.464**	1.412**
Root Length	48.043**	300.313**	11.778**
Fresh Shoot Weight	49.486**	4713.883**	47.712**
Fresh Root Weight	33.512**	224.16**	5.316**
Excised leaf water loss	1291.882**	1495.816**	1050.464**
Proline	260.741**	23926.133**	102.7**
Sugar	145.219**	14057.016**	58.221**
Glycine Betaine	59.76**	4862.937**	28.938*
Cell Membrane Stability	998.603**		
Osmotic Adjustment	0.185**		

*= Significant
** = Highly Significant
^{ns} = Non-significant

Table 2. Phenotypic and genotypic correlation of thirty wheat genotypes for physiological and growth traits under drought stress.

Variables	Shoot Length	Root Length	Fresh Shoot Weight	Fresh Root Weight	Excised Leaf Water Loss	Proline	Sugar	Glycine Betaine	Cell Membrane Stability	Osmotic Adjustment
Shoot Length		-0.5008**	0.0766	0.0979	0.0556	-0.0383	-0.0374	-0.0454	-0.3973*	0.1413
Root Length	-0.3221		0.4292*	-0.1034	0.1499	-0.2071	-0.2046	-0.2443	0.7814**	-0.1550
Fresh Shoot Weight	0.1472	0.2821		0.0245	0.2044	0.5287**	0.5352**	0.4708**	0.7117**	0.4056*
Fresh Root Weight	0.0520	-0.0624	0.0799		-0.1430	-0.3033	-0.3132	-0.2029	-0.1405	-0.1592
Excised Leaf Water Loss	0.0314	0.0947	0.1297	-0.1246		0.2464	0.2429	0.2590	0.6187**	-0.0057
Proline	0.0201	0.0376	0.2351	-0.2025	0.1517		1.0000	1.0000	0.3141	0.8314**
Sugar	0.0203	0.0432	0.2296	-0.2099	0.1499	0.9993**		1.0000	0.3073	0.8286**
Glycine Betaine	0.0248	-0.0061	0.2660	-0.1171	0.1457	0.9447**	0.9318**		0.3624*	0.8675**
Cell Membrane Stability	-0.2257	0.3229	0.2328	-0.1115	0.3752*	0.1649	0.1704	0.1013		0.1764
Osmotic Adjustment	-0.0589	0.0052	0.1887	-0.1002	0.0016	0.5359**	0.5384**	0.4821**	-0.0406	

*Values presented above diagonal is genotypic correlations and below diagonal is phenotypic correlations, *= Significant, ** = Highly Significant

Table 3. Phenotypic and genotypic correlation of thirty wheat genotypes for physiological and growth traits under control (well water conditions).

Variables	Shoot Length	Root Length	Fresh Shoot Weight	Fresh Root Weight	Excised Leaf Water Loss	Proline	Sugar	Glycine Betaine	Cell Membrane Stability	Osmotic Adjustment
Shoot Length		0.0192	0 .5562**	0.1146	-0.0399	-0.0974	-0.1166	-0.0943	-0.7247**	-0.0703
Root Length	0.6750		0.1421	-0.4935**	0.4913**	0.1419	0.1554	0.1467	0.3269	0.0016
Fresh Shoot Weight	0.3193	0.1824		0.2888	0.0366	-0.1401	-0.1167	-0.1421	-0.3535	-0.3711*
Fresh Root Weight	0.1012	-0.3761*	0.2388		-0.4871**	-0.3377	-0.2987	-0.3427	-0.3294	-0.2659
Excised Leaf Water Loss	-0.0295	0.3377	-0.0127	-0.3044		0.2107	0.2255	0.2235	0.3885*	0.3688*
Proline	0.0317	0.0784	0.1471	-0.1280	0.1210		1.0023	0.9998**	0.1876	0.0332
Sugar	0.0252	0.0857	0.1362	-0.1272	0.1093	0.9954**		1.0019**	0.1681	0.0376
Glycine Betaine	0.0323	0.0798	0.1463	-0.1301	0.1235	1.0000**	0.9954**		0.1902	0.0396
Cell Membrane Stability	-0.2670	0.1736	-0.0968	-0.2020	0.0481	0.2353	0.2297	0.2359		0.1764
Osmotic Adjustment	-0.0797	-0.0561	-0.2176	-0.1538	0.1093	-0.3870*	-0.3935*	-0.3855*	-0.0406	

*Values presented above diagonal is genotypic correlations and below diagonal is phenotypic correlations, *= Significant, ** = Highly Significant

Genotypic correlation results revealed that shoot length had highly significant negative correlation with root length and significant negative with cell membrane stability under drought stress. This may be due to better portioning of carbon cost towards the root elongation and membrane thickness under drought stress, in agreement with general consensus that under water shortage condition tolerant genotypes elongate their roots into deep soil zones to extract soil moisture efficiently (Wasson *et al.*, 2012; Narayanan *et al*., 2014). Under control (well-water conditions) the traits shoot length and fresh shoot weight showed highly significant positive correlation whereas highly significant negative correlation prevailed between shoot length and cell membrane stability. Root length showed highly significant and significant positive correlation with cell membrane stability and fresh shoot weight respectively, under drought stress. Whereas under control (well-water conditions), root length exhibited highly significant positive correlation with excised leaf water loss. While highly significant negative correlation existed among root length and fresh root weight. Fresh shoot weight exhibited the highly significant positive correlation with various traits including; proline, sugar, glycine betaine and cell membrane stability while significant positive osmotic adjustment under drought stress. Fresh shoot weight also established the significant negative correlation with the osmotic adjustment under control (well-water conditions). The highly significant positive correlation presented between the traits; excised leaf water loss and cell membrane stability under drought stress. Similarly under control (well-water conditions), excised leaf water loss revealed significant positive correlations with cell membrane stability and osmotic adjustment. These correlations appeared might be not of routine. Proline and sugar contents had highly significant positive correlation with osmotic adjustment under drought stress. Likewise the both traits i.e. proline and sugar showed the highly significant correlation with glycine betaine under control (well-water conditions). While under drought stress, the glycine betaine demonstrated the highly significant and significant positive correlations with osmotic adjustment and cell membrane stability respectively. These results gave the support of idea that the accumulation of osmolytes under drought stress helps plant to avoid stress by enhancing the osmotic adjustment capacity of plants as described by many studies (Oraki *et al*., 2012; Raza *et al*., 2012; Naeem *et al*., 2015).

Phenotypic correlation results revealed that root length had significant negative correlation with fresh root weight under control (well-water conditions). Excised leaf water loss showed the significant positive correlation with cell membrane stability under drought stress. The trait proline showed the highly significant positive correlation with various traits including sugar, glycine betaine and osmotic adjustment under drought stress. While under control (well-water conditions), proline revealed the highly significant positive correlation with sugar, glycine betaine, whereas significant negative correlation with osmotic adjustment. Similarly, glycine betaine revealed the highly significant positive correlation with osmotic adjustment under drought stress, while opposite behavior showed under control (well-water conditions) that glycine betaine had significant negative correlation with osmotic adjustment.

Results formulated that with the intensive selection of root length, fresh shoot weight, proline, sugar, glycine betaine, cell membrane stability and osmotic adjustment will ultimately improve the drought tolerance. Since these seven traits are correlated with each other, so selection in one of the above mentioned traits will results in the improvement of other traits. Highly significant and negative correlation of shoot length were with root length was observed under drought stress. This correlation indicating that more preference should be given to long rooted varieties as they could be efficiently extract the moisture in deeper soil, hence root length trait could be used to the advantage in direct selection for drought tolerance.

Path Analysis for Osmotic Adjustment: Correlation studies could not evaluate the indirect effects of traits on osmotic adjustment. Path coefficient analysis provided an effective way of finding out direct and indirect effects and permitted a critical examination of the specific forces acting to produce a given correlation. The path analysis gave a clue to the contribution of various growth and physiological traits on osmotic adjustment in the genotypes under study. The growth traits such as shoot length, root length, fresh shoot weight, fresh root weight and physiological traits such as excised leaf water loss, proline, sugar, glycine betaine, cell membrane stability were considered as independent variables and path analysis was employed in order to evaluate the effect and contribution of these components on dependent variable which is osmotic adjustment.

The positive direct effects of five characters viz. shoot length (1.1053), root length (1.8386), fresh root weight (0.4742), sugar (3.3994), glycine betaine (1.1224) on osmotic adjustment exhibited that under drought stress (Table 4) rather than the control (well water condition) where all traits have negative direct effect on osmotic adjustment (Table 5). These results were in agreement with general consensus that osmotic adjustment was enhanced under stress condition, where different growth and physiological traits were influenced and activated, helped the plants to avoid the stress condition by initiating osmotic adjustment strategy. Physiological traits like sugar (3.3994), glycine betaine (1.1224) and growth related traits like shoot length (1.1053), root length (1.8386) and fresh root weight

(0.4742) had positive direct effect under drought stressed environment. This suggested that these physiological and growth traits under drought stress were greater influenced to avoid the drought stress by adopting osmotic adjustment strategy.

Table 4. Direct and indirect effects of physiological and growth traits on osmotic adjustment under drought stress.

Variables	Shoot Length	Root Length	Fresh Shoot Weight	Fresh Root Weight	Excised Leaf Water Loss	Proline	Sugar	Glycine Betaine	Cell Membrane Stability	Osmotic Adjustment
Shoot Length	1.1053	-0.9208	-0.1032	0.0464	-0.0205	0.0851	-0.1270	-0.0510	0.1268	0.1413
Root Length	-0.5535	1.8386	-0.5779	-0.0490	-0.0554	0.4609	-0.6956	-0.2742	-0.2495	-0.1550
Fresh Shoot Weight	0.0847	0.7892	-1.3464	0.0116	-0.0755	-1.1767	1.8194	0.5284	-0.2272	0.4056
Fresh Root Weight	0.1082	-0.1902	-0.0330	0.4742	0.0528	0.6750	-1.0645	-0.2278	0.0449	-0.1592
Excised Leaf Water Loss	0.0614	0.2755	-0.2753	-0.0678	-0.3695	-0.5483	0.8259	0.2907	-0.1975	-0.0057
Proline	-0.0423	-0.3808	-0.7118	-0.1438	-0.0910	-2.2257	3.3998	1.1308	-0.1003	0.8314
Sugar	-0.0413	-0.3762	-0.7206	-0.1485	-0.0898	-2.2260	3.3994	1.1331	-0.0981	0.8286
Glycine Betaine	-0.0502	-0.4492	-0.6339	-0.0962	-0.0957	-2.2423	3.4318	1.1224	-0.1157	0.8675
Cell Membrane Stability	-0.4391	1.4367	-0.9583	-0.0666	-0.2286	-0.6990	1.0448	0.4068	-0.3192	0.1764

Table 5. Direct and indirect effects of physiological and growth traits on osmotic adjustment under control (well water condition).

Variables	Shoot Length	Root Length	Fresh Shoot Weight	Fresh Root Weight	Excised Leaf Water Loss	Proline	Sugar	Glycine Betaine	Cell Membrane Stability	Osmotic Adjustmen t
Shoot Length	-0.1570	-0.0022	-0.2756	0.0098	-0.0243	-0.5458	0.1722	0.3978	0.0407	-0.0703
Root Length	0.0030	-0.1164	-0.0704	-0.0421	0.2993	0.7950	-0.2294	-0.6190	-0.0184	0.0016
Fresh Shoot Weight	0.0873	-0.0165	-0.4954	0.0246	0.0223	-0.7852	0.1722	0.5996	0.0199	-0.3711
Fresh Root Weight	0.0180	0.0574	-0.1431	-0.0852	-0.2967	-1.8922	0.4414	1.4456	0.0185	-0.2659
Excised Leaf Water Loss	-0.0063	-0.0572	-0.0182	-0.0415	-0.6091	1.1805	-0.3330	-0.9429	-0.0218	0.3688
Proline	-0.0153	-0.0165	0.0694	-0.0288	0.1283	-5.6039	-1.4799	-4.2174	-0.0105	0.0332
Sugar	-0.0183	-0.0181	0.0578	-0.0255	0.1374	5.6166	-1.4765	-4.2263	-0.0095	0.0376
Glycine Betaine	-0.0148	-0.0171	0.0704	-0.0292	0.1362	5.6025	-1.4793	-4.2185	-0.0107	0.0396
Cell Membrane Stability	-0.1138	-0.0380	0.1751	-0.0281	0.2366	1.0515	-0.2483	-0.8025	-0.0562	0.1764

In drought stressed environment, path coefficient analysis for osmotic adjustment showed that the traits like sugar (3.3994), glycine betaine (1.1224) demonstrated highest positive direct effect on osmotic adjustment. The high positive direct effect of sugar and glycine betaine was responsible for its significant positive correlation with osmotic adjustment. This indicated that a slight increase one/or both of the above traits may directly contribute to osmotic adjustment. The highest positive indirect effect towards the osmotic adjustment was exhibited by shoot length via cell membrane stability (0.1268); root length via proline (0.4609); fresh shoot weight via sugar (1.8194) followed by root length (0.7892); fresh root weight via proline (0.6750); excised leaf water loss via sugar (0.8259); proline via sugar (3.3998) and glycine betaine (1.1308); sugar via glycine betaine (1.1331); glycine betaine via sugar (3.4318); cell membrane stability via root length (1.4367) and followed by sugar (1.0448). In control (well water condition), path coefficient analysis for osmotic adjustment showed that all traits have negative direct effect towards osmotic adjustment. The negative direct effect on osmotic adjustment revealed that drought escaping mechanism i.e. osmotic adjustment was switched off under control (well water condition).

On the basis of path coefficient analysis under drought stress, the maximum indirect effects of sugar, root length and proline were on osmotic adjustment. Hence, it may be concluded that sugar, root length and proline are the main traits which are responsible for the exploitation of osmotic adjustment in wheat. Selections on the basis of these traits for high osmotic adjustment are more effective under drought stress condition.

Conclusion: Results of present study concluded that the traits including root length, fresh shoot weight, proline, sugar, glycine betaine, cell membrane stability and osmotic adjustment revealed significant positive correlations among these traits, suggested the considerable prospects for improvement under drought stress. Therefore, intensive selection on the basis of these traits in transgressive segregants will ultimately improve the drought tolerance. Highly significant negative correlation among shoot length and root length implicates that scientists should gave consideration on long rooted genotypes under drought stress. The path analysis indicated that the traits sugar and root length and glycine betaine had high direct and indirect effects on osmotic adjustment under drought stress. Selection on the basis of these traits is of great importance for cultivar development with considerable drought tolerance at early growth stage.

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