Research Article

Genotypic Variability and Association between Seed Traits and Seedling Vigor in Upland Cotton

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Abstract | Physical attributes of seed play a prime role in seedling establishment. Present study was conducted to investigate genotypic variability and correlations among upland cotton (*Gossypium hirsutum*) cultivars for some physical (seed weight, seed surface area and seed density) and biochemical (seed oil contents) seed traits. The study determined the effects of water stress on genetic variability of root and shoot traits and association of the seed traits with seedling vigor. 15 cotton cultivars were studied at two levels of irrigation during 2014-15. Results showed highly significant ($p \leq 0.05$) genotypic differences for seed and root–shoot traits. Reduction in fresh weight of different cultivars due to water stress ranged 14.74 to 29.81% and reduction in dry weight due to water stress range 12.52 to 43.30%. Whereas increase in root-shoot ratio was observed due to water stress and it ranged from 11.19 to 54.05% for different genotypes. Cultivar into irrigation interaction was significant and genotypic variability declined under water stress conditions relative to irrigated conditions. Fresh and dry shoot weights were positively correlated with all seed traits. The highest correlation (0.94) was observed between seed weight and fresh shoot weight under irrigated condition followed by correlation of dry shoot weight with seed weight (0.89) under irrigated conditions. Correlation of all the studied traits decreased under water stress conditions. Correlation of root-shoot ratio with seed weight (0.19), seed surface area (0.49) and seed density (0.17) was positive under irrigated conditions but changes to negative (-0.41, -0.28, -0.39 respectively) under water stress conditions. A highly significant correlation between seed physical traits and seedling vigor showed possibility of quick selection for plant breeders on the basis of seed traits.

Received | November 12, 2018; **Accepted** | March 13, 2020; **Published** | June 22, 2020
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Citation | Iqbal, M., S.U. Allah, M. Naeem, M. Ijaz and M.Q. Ahmad. 2020. Genotypic variability and association between seed traits and seedling vigor in upland cotton. *Pakistan Journal of Agricultural Research,* 33(2): 337-343.
DOI | http://dx.doi.org/10.17582/journal.pjar/2020/33.2.337.343
Keywords | Biochemical chrastristics, Correlation, Cotton seed, Root shoot traits

Introduction

Cotton is the most important cash crop of Pakistan, which is mostly grown under hot climate. Increasing scarcity of irrigation water in Pakistan may severely affect cotton production (Kirby et al., 2017; Qureshi, 2018). A wide genotypic variation has been reported for drought tolerance in cotton cultivars developed in Pakistan (Ullah et al., 2008; Rahman et al., 2008; Iqbal et al., 2017) and in the world (Bardak and Bolek, 2012); therefore, there is a need to exploit this variation for sustainable production.

Previous studies found a strong relationship between seedling traits and final yield of cotton (Irum et al., 2013; Ul-Allah et al., 2017). Bednarz et al. (2005)

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emphasised management of the most basic yield components to improve seed cotton yield. Wanjura et al. (1969) reported a strong relationship of time to emergence with seed quality, planting depth and seed cotton yield. Leffler and Williams (1983) reported a close relationship of seedling growth and germination index of cotton with the seed density. Cook and Zik (1992) reported significant correlation between root-shoot ratio and drought induced flower abscission in cotton. Irum et al. (2013) reported strong association among root-shoot traits of cotton. Snider et al. (2016) reported a strong association of seed weight and seed oil contents with seedling vigor. These relationships can be exploited to evaluate the crop plants at early stages of crop growth that make genotypic selection procedure rapid and cost-efficient.

Germination and initial growth are dependent on seed nutritive reserve until photosynthesis begins (Turley and Chapman, 2010). A cotton variety with better seed physical traits (seed weight, seed volume, seed surface area, seed density etc.) may also have better seed reserves (Pahlavni et al., 2008). A positive correlation between seed oil content and seedling vigor in cotton has been reported by Snider et al. (2104) and Pahlavani et al. (2008). Seed quality is an important factor for seedling establishment (Pahlavani et al., 2008) and seedling vigor may contribute significantly to deal with stress at early stages of crop growth. An adequate information on relationship of seedling characteristics and seed physical and chemical traits in cotton is available (Khan et al., 2004; Pahlavani et al., 2008; Irum et al., 2013) but there are only a few studies that describe relationship of seed traits with seedling vigor in upland cotton (Snider et al., 2014). Significant effects of cultivar and environment on seed traits have been reported (Ye et al., 2003; Song and Zhang, 2007), but there are hardly any studies that describe seed traits of the varieties developed in semi-arid and sub-tropical climate in relation to seedling vigor. Main objectives of the study were to determine genotypic variability for physical (seed volume, seed index, seed density, seed weight and seed surface area) and biochemical seed trait (seed oil contents) measured in upland cotton cultivars and to study the effects of water stress on genetic variability of root-shoot traits and their association with seed traits and seedling vigor.

Materials and Methods

The experiment was conducted in a completely randomized design (CRD) with three replications in November 2014. The greenhouse day/night temperature was 35/28 °C and relative humidity 40/60%. Seeds of 15 commercial cotton cultivars (Table 2) were obtained from the Department of Plant Breeding and Genetics, University College of Agriculture and Environmental Sciences, The Islamia University, Bahawalpur, Pakistan and were planted in black colored polythene bags [75 cm length × 12 cm width; filled with sandy soil (70% sand, 5% clay and 25% silt)]. Seed of each cultivar was taken from single source (same breeding institute) to measure seed volume, seed weight, seed size, seed density and seed oil content. Uniform seed were used to avoid any differences due to seed physical traits, which may affect characterization and correlation of seed physical/ chemical properties with seedling vigor. Four seeds were sown in each bag; stand was thinned to a single seedling per bag after germination. Under irrigation treatment, plants were supplied 150 ml water daily; whereas, water stressed plants were given 150 ml of water for the first 10 days; after that watering was done at 4-d intervals. The bags were fertilized with two gram each of nitrogen and phosphorus 10 days after sowing for proper nutrition of the seedlings. Data for shoot–root traits were recorded 30 days after sowing.

For seed properties, data were recorded for seed weight, seed volume, seed surface area, seed density and seed oil content. Three samples of 100 seeds from each cultivar (each sample as one replication) were delinted with sulfuric acid (conc. 55%), followed by rinsing with tap water until all acid was removed. The samples were then surface dried, followed by drying in an oven at 40 °C for 48 hours and then seed weight of 100 seed was recorded. From the same seed sample, seed volume was calculated by ethanol displacement method (Groves and Bourland, 2010) using a 30 ml graduated jar. Seed density was calculated by using the following formula (Groves and Bourland, 2010):

$$\text{Seed density} = \frac{\text{Seed weight}}{\text{Seed volume}}$$

Seed surface area was calculated according to Hodson (1920) which is based on volumetric displacement of ethanol by acid delinted cotton seed with assumption that all seeds have uniform shape. Cotton seed oil content was determined using the Soxhlet procedure (AOAC, 1990) by using hexane solvent at 60 °C followed by removal of extra solvent by refluxing at 70 °C. 0.5M sodium hydro oxide was used to clarify the crude cotton seed.

After 25 days of emergence, seedlings were harvested from polythene bags followed by washing gently with tap water. seedlings were spread on paper to measure their shoot and root length. Fresh weights of roots and shoots were measured. Shoots were dried at 65 °C for 72 hours and dry shoot weight was measured.

Data were analyzed by using analysis of variance (ANOVA) using MSTAT-C computer software (Russell, 1994) for genotypic comparison followed by Tukey's comparison test to compare the means. Correlations between seed traits and seedling vigor traits were calculated according to Gomez and Gomez (1984).

Results and Discussion

The analysis of variance showed significant differences for all measured seed characteristics, viz., 100 seed weight, 100 seed volume, seed density and seed oil content among cultivars (Table 1). Seed volume ranged from 6.23 to 9.20 ml, seed weight ranged from 6.07 to 8.80 g, seed surface area ranged from 0.31 to 0.43 mm^2, seed density ranged from 0.78 to 1.26 g ml^{-1} and seed oil content ranged from 196.42 to 236.20 g kg^{-1} (Table 2).

Table 1: *Genotypic mean squares of seed traits of cotton cultivars before sowing and seedling parameters of cotton cultivars analysed under different planting conditions as obtained in Bahawalpur, Pakistan during 2014-15.*

Seed and seedling traits	Degrees of freedom	Mean squares	
Seed traits			
Seed volume	14	1.270**	
Seed Weight	14	1.032**	
Seed size	14	0.29**	
Seed density	14	0.067**	
Oil contents	14	40.80**	
Seedling traits			
		İrrigated	Water stressed
Fresh weight	14	0.378**	0.358**
Dry weight	14	0.056**	0.072**
Root-shoot ratio	14	0.196**	0.043*

**, **Significant at 0.05 and 0.01 probability level, respectively.*

Table 2: *Genetic variability in seed traits for different cotton cultivars grown in Pakistan as obtained in Bahawalpur, Pakistan during 2014-15.*

Cultivar	100 Seed volume (ml)	100 Seed weight (g)	Seed surface (mm^2)	Seed density (g ml^{-1})	Oil contents (g kg^{-1})
MNH-886	6.23^{e†}	7.86^{c}	0.98^{bc}	1.26^{a}	224.24^{c}
IUB-222	7.53^{bc}	8.40^{ab}	0.92^{cd}	1.11^{ab}	234.31^{ab}
CIM-534	8.40^{ab}	6.92^{e}	0.93^{cd}	0.82^{cd}	216.06^{de}
CIM-707	7.93^{b}	6.33^{f}	0.97^{bc}	0.79^{cd}	208.09^{f}
FH-142	6.52^{d}	7.91^{c}	0.99^{b}	1.21^{a}	228.53^{bc}
Sitara-008	6.32^{e}	6.40^{f}	0.95^{c}	0.72^{d}	209.14^{ef}
Sitara-09	8.92^{ab}	7.00^{e}	0.92^{cd}	0.78^{cd}	216.70^{d}
FH-113	7.89^{b}	6.86e^{f}	0.93^{cd}	0.86^{c}	209.05^{f}
CIM-598	6.53^{d}	6.07^{g}	0.95^{c}	0.92^{bc}	224.52^{c}
NS-131	6.75^{cd}	7.50^{cd}	0.94^{c}	1.11^{ab}	229.24b^{c}
N-111	8.72^{ab}	8.80^{a}	0.97b^{c}	1.01^{b}	236.20^{a}
CIM-473	7.62^{bc}	7.30^{d}	0.95^{c}	0.95^{bc}	217.10^{d}
MNH-786	9.20^{a}	7.20^{d}	0.103^{a}	0.78^{cd}	216.14^{de}
IR-3701	6.91^{cd}	6.39^{f}	0.92^{cd}	0.92^{bc}	199.40^{g}
NIBGE-115	7.23^{c}	6.24^{fg}	0.90^{d}	0.86^{c}	196.42^{g}

†Means sharing the same letter(s) in each column do not differ at 0.05 probability level.

Seedling vigor was estimated in the greenhouse under irrigated and water stress conditions. Mean values of root-shoot traits are given in Table 3. Under both irrigated and stress conditions, cv. N-11 had the highest fresh weight (3.27 and 2.85g, respectively), followed by IUB-222 (3.21 and 2.65g, respectively). CIM-707 and CIM-598 recorded 31.8 and 29.8% reduction in fresh weight because of water stress; the lowest reduction in fresh weight (14.7%) was recorded in cultivar (cv.) N-111.

Under irrigated conditions, IUB-222 obtained the highest dry weight (0.39 g), followed by N-111 (0.37 g); under stress conditions, N-111 had the highest dry weight (0.34 g), followed by Sitara-09 (0.29 g). The largest reduction in dry weight under water stress conditions compared to the full irrigation treatment was in NIBGE-115, i.e., 43.1%; whereas the lowest reduction occurred in N-111, i.e., 4.5%. Root-shoot ratio is considered an important parameter for evaluation of drought stress. Under irrigated conditions, FH-142 had the highest root-shoot ratio (1.68), whereas under stress conditions, IUB-222 had the highest value (1.49), followed closely by N-111 (1.48).

Table 3: *Seedling vigor performance of different cultivars studied under greenhouse (irrigated and water stress) as obtained in Bahawalpur, Pakistan during 2014-15.*

Cultivars	Fresh weight (g)			Dry weight (g)			Root-shoot ratio (Length)		
	Irrigat-ed	Water stressed	% decrease in water stress	Irrigated	Water stressed	% decrease in water stress	Irrigated	Water stressed	% decrease in water stress
MNH-886	2.83^{de†}	2.29^{d}	23.58	0.29^{cd}	0.23^{c}	25.19	0.71^{h}	1.17^{c}	-39.32
IUB-222	3.21^{b}	2.63^{b}	22.05	0.39^{a}	0.30^{ab}	30.00	0.75^{ef}	1.24^{bc}	-39.52
CIM-534	2.44^{i}	2.05^{h}	19.02	0.27^{d}	0.21c^{d}	31.30	0.82^{def}	1.30^{abc}	-36.92
CIM-707	2.32^{j}	1.76^{j}	31.82	0.25^{e}	0.20^{d}	29.21	0.77^{ef}	1.22^{c}	-36.89
FH-142	2.95^{c}	2.53^{c}	16.60	0.29^{cd}	0.24^{c}	20.83	1.68^{a}	1.19^{c}	-29.17
Sitara-008	2.54^{h}	2.01^{h}	26.37	0.26^{de}	0.20^{d}	30.02	0.85^{cde}	1.22f^{c}	-30.33
Sitara-09	2.81^{e}	2.25^{de}	24.89	0.32^{c}	0.25^{bc}	28.14	0.79^{def}	1.22^{c}	-35.25
FH-113	2.75^{f}	2.16^{f}	27.31	0.33^{bc}	0.27^{b}	23.53	0.93^{cd}	1.30^{abc}	-28.46
CIM-598	2.09^{l}	1.61^{l}	29.81	0.28^{d}	0.21^{cd}	31.11	0.68^{f}	1.47^{ab}	-54.05
NS-131	2.88^{d}	2.24^{e}	28.57	0.30^{cd}	0.25b^{c}	42.81	0.75^{ef}	1.48^{a}	-49.32
N-111	3.27^{a}	2.85^{a}	14.74	0.36^{b}	0.32^{a}	12.52	0.83^{de}	1.31^{abc}	-36.64
CIM-473	2.69^{g}	2.13f^{g}	26.29	0.27^{d}	0.21^{cd}	27.05	0.77^{ef}	1.49^{a}	-48.32
MNH-786	2.68^{g}	2.12^{g}	26.42	0.30^{cd}	0.24^{c}	27.78	1.19^{b}	1.34^{abc}	-11.19
IR-3701	2.29^{j}	1.85^{i}	23.78	0.28^{d}	0.22^{cd}	23.67	0.73^{ef}	1.37^{abc}	-46.72
NIBGE-115	2.17^{k}	1.71^{k}	26.90	0.27^{d}	0.19^{e}	43.30	0.98^{c}	1.54^{a}	-36.36

†Means sharing the same letter(s) in each column do not differ at 0.05 probability level.

Table 4: *Correlation of seed traits with fresh weight, dry weight and root-shoot ratio under irrigated and water stress conditions as obtained in Bahawalpur, Pakistan during 2014-15.*

Seed parameters	Irrigated			Under water stress		
	Fresh weight (g)	Dry weight (g)	Root-shoot ratio (Length)	Fresh weight (g)	Dry weight (g)	Root-shoot ratio (Length)
Seed volume (ml)	0.29*	0.30*	0.01	0.03	0.13	0.04
Seed weight (g)	0.94**	0.89**	0.19	0.74**	0.79**	-0.41**
seed surface Area (mm^2)	0.23	0.21	0.49**	0.39**	0.41**	-0.28*
Seed density (g ml^{-1})	0.52**	0.56**	0.17	0.45**	0.51**	-0.39*
Oil contents (g kg^{-1})	0.58**	0.63**	0.19	0.49**	0.59**	0.11

,Significant at 0.05 and 0.01 probability level, respectively.*

Under irrigated conditions, seed weight, seed density and oil content had significant (P<0.01) positive correlations with fresh and dry shoot weight; the correlation of seed surface area with seed volume was significant at P<0.05. The highest numerical value of correlation was observed for seed weight with fresh weight (r=0.94), followed by dry shoot weight (r=0.89). Root-shoot ratio under irrigated conditions showed a highly significant correlation (r=0.49; P<0.01) only with seed surface area. Under water stress conditions in the greenhouse experiment, all seed traits, except seed volume and seed surface area, showed significant (P<0.01) positive correlations with fresh and dry weight. Under water stress, the correlation of seed weight with fresh weight was 0.74 and that with dry weight was 0.79. The magnitude of the correlation was reduced under the water stress conditions. Maximum reduction in in correlation due to water stress was found between seed volume and fresh weight which reduced 0.29 under irrigated to 0.03 under water stress (Table 4). Correlation of root-shoot ratio was significant and negative with seed weight (-0.41; P<0.01), seed surface area (-0.28; P<0.05) and seed density (0.39; P<0.05) under water stress conditions (Table 4).

Physical seed traits are very important in estimating the seedling and plant vigor under drought stress as they are directly related to seed health. Seed characteristics (seed weight, seed volume, seed density, seed surface area) have been reported to have a significant role in plant development (Snider et al., 2016). A large range of variation existed among the cultivars for traits such as seed volume, seed weight, seed surface area, seed density and seed oil content (Snider et al., 2014; Zeng et al., 2007), which suggested the possibility of selection of genotypes on the basis of seed traits.

Root-shoot parameters are considered important in estimating cultivar performance on the bases of seedling vigor. Significant correlation of root–shoot traits of cotton with the final yield have long been known (Irum et al., 2013; Cook and El–Zik, 1992). All cultivars showed an increase in root–shoot ratio under water stress conditions. This increase in root-shoot ratio was the result of an increase in root length, in search of water, under water stress conditions (Pace et al., 1999; Ferreira et al., 2013). All other root-shoot traits decreased under water stress, as water stress limited the supply of nutrients to the growing vegetative parts. Jamal et al. (2014) reported a lower reduction in root–shoot traits as compared to our results; a possible reason for this might be that Jamal et al. (2014) used diploid species of cotton and we used tetraploid species. Increase in root length under water stress showed the ability of plant to manage water requirements. Increase in root length might be attributable to the activation of some genes under the stress condition (Vadez et al., 2013) and bio-physiological processes like antioxidant enzymatic activities which promoted root growth (Pace et al., 1999). Hsiao and XU (2000) reported differential response of root and shoot to water stress, which was attributable to the ability of roots to adjust osmotic potential under water stress conditions. As the performance of IUB-222 and N-111 relative to fresh and dry weight under greenhouse (water stress and irrigated conditions) improved, they were regarded as drought tolerant cultivars.

Significant correlation of seedling traits (root-shoot weight and ratio) with seed characteristics (seed volume, seed weight, seed density and seed surface area) showed that seed reserves influenced crop growth at early stages that are most susceptible to water stress (Snider et al., 2014; Nik et al., 2011; Doman et al., 1982). Correlation in our study is weaker than that reported by Snider et al. (2014) and the reason might be differences in day/night temperature and humidity between the two studies, as seedling growth is much affected by these factors (Jaleel et al., 2009). Strength of correlation decreased under water stress conditions, which might be attributable to reduced variability among the cultivars under the stress conditions, as observed in the current study. Reduction in strength of association of plant characters under stress conditions was comparable to other researchers as it has also been reported by Khan et al. (2004) in maize (*Zea mays* L.) and Rahman et al. (2008) in cotton. The correlation of root–shoot ratio with seed traits was positive and significant under irrigated conditions, but it was negative under water stress conditions (Table 4). A possible reason can be that seed physical properties contributed same to root and shoot traits under both condition, but under water stress conditions, root length increased because of water stress response of plant physiological and biochemical processes (Pace et al., 1999), which might be responsible for the negative relationship.

Conclusions and Recommendations

Significant genotypic differences existed for seed and seedling traits among cotton cultivars. Cultivars IUB–222 and N–111 performed the best under irrigated conditions because of their genetic makeup. Highly significant correlations of seed weight, seed size and seed oil content with seedling vigor showed the possibility of selection on the basis of these seed traits. As vigorous seedlings can withstand early stress more efficiently, use of high-quality seeds is required. Care should be taken in selection, as direction of association turned negative under water stress conditions. Cotton breeder should consider seed physical traits for selection of genotypes under stress conditions. Similarly, famers may also consider seed physical traits in choosing crop variety especially under water stress conditions. As correlation values changed under various environments, therefore association of the traits must be reconfirmed under specific environmental conditions.

Author's Contribution

M. Iqbal conceived idea and conducted experiment and technical input at every step, S. UL-Allah wrote Introduction, Results and Discussion, Finalized first draft. M. Naeem wrote abstract and methodology. M. Ijaz wrote introduction and did critical revision and M.Q. Ahmed wrote discussion and did critical revision.

Conflict of interest

The authors have declared no conflict of interest.

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