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Correlations among physical, physiological and biochemical seed traits in sunflower hybrids

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Abstract
The seed traits like physical, physiological and biochemical are complex and knowledge on trait correlations would be helpful in knowing the relations among them in improving seed quality and breeding purposes. In this study, correlations among seed traits were studied and compared between five hybrids of sunflowers i.e., GK 202, KBSH 44, KBSH 53, RSFH 130 and RSFH 1887. Some previously published correlations in hybrids or varieties could be redetected and some disappeared, which supported the hypothesis that correlations in hybrids could be 'weaker or incidental' or stronger. Physical seed traits relationships suggest that germination, field emergence and speed of germination content can be improved by seed size (seed thickness, length breadth thickness ratio) traits and may be decreased through increase in bulk density. Reducing the protein content without affecting the other traits can able to increase the germination. As test weight is related to dehydrogenase activity, indirectly it plays a role in germination, dry weight, seedling vigour indexes as well as hull content. These traits are not correlated with bicohemical traits like electrical conductivity, catalase and peroxidase activity. The results obtained from correlation showed that the efficiency in the improving physiological status of hybrids in sunflower should increased through physical and biochemical seed traits.

Keywords: physical, physiological, biochemical, hybrid

Introduction
Sunflower (*Helianthus annuus* L.) is an edible oilseed crop grown after soybean and groundnut. Its seeds are economically important, contain valuable edible oil that contains more Vitamin E than any other vegetable oil. It is used in the manufacturing paints, resins, plastics, soap, cosmetics and oil cake is rich in high quality protein (40–44%) and is used as cattle and poultry feed. India accounts for 0.69 million ha of area and 0.54 million tonnes of production. Maharashtra, Karnataka, Telangana and Andhra Pradesh are the major sunflower growing states. Seed yield (~793 Kg/ha) and oil is a rich source of lenoleic acid (64%) and 25-30 per cent of oleic acid which helps in washing out cholesterol deposition in the coronary arteries of the heart and thus is good for heart patients. Relations between physical, physiological and biochemical status of seeds would eventually lead to increase in other useful quality traits. The potential of cultivars can be realized when beneficial seed traits *per se* combines positively with seed physical, physiological and biochemical traits. Therefore, the relationships among physical, physiological and biochemical traits is very essential to identify a combination of traits to be used for selection in breeding programmes of sunflower.

Materials and Methods
Five sunflower hybrids *ie.*, GK-202, KBSH-44, KBSH-53, RSFH-130 and RSFH-1887 were used in this study and were maintained at Main Agricultural Research Station, University of Agricultural Sciences, Raichur, 584104. A range of seed traits were measured using the procedures described below.

Physical traits
Seed size (mm)
Seed length, width and thickness were measured for 10 randomly selected seeds with digital grain vernier meter and the average value was expressed in milli meters. Seed size was also defined as the ratio between length and breadth (Length to Breadth Ratio) and as the product of maximum seed length and maximum seed width and maximum seed thickness (Alexander *et al*. 2001) [3].

Test weight (g)
Randomly selected 100 seeds were weighed on a top pan balance with an accuracy of 0.001g and the average value was expressed in g.

Bulk density (Kgm^{-3})
Bulk density is the ratio of mass sample of seed to its total volume was determined by filling a 1000 ml container with kernels from a height of about 15 cm, striking the top level and then weighing the content (Deshpande 1993) [5].

Hull content (%)
The percentage of the fraction of hulls was calculated as the ratio of the seed hull to the total seed. Hundred seeds per seed sample per replication were dried (5 hat 60^{0}C), weighed and afterwards germinated for 15 h in water. The seed hulls were separated, further dried (5 hat 60^{0}C) and weighed (Rao *et al*., 1977). Fraction of hull was worked out as hull weight divided by whole seed weight and expressed in percentage.

Physiological Traits
Standard germination (%) was conducted on pure seed fraction using 100 seeds in four replicates following between paper (BP) method at 250C temperature and 93±2 per cent relative humidity. The numbers of normal seedlings were counted on 4th day (first count) and 10th day (final count) of germination from all the replications. It was worked out as total number of normal seedlings divided by total number of seeds kept for germination and expressed in percentage.
Mean seedling length (cm) was done taken by Ten normal seedlings were taken at random in each treatment and replication at the time of final count of germination test. The shoot and root lengths were measured from collar region to point of attachment of cotyledons and from the collar region to the tip of primary root, respectively. Sum total of the shoot and root lengths constitute total length of seedling. The mean seedling length in each treatment and replication was calculated and expressed in centimeters.

Mean seedling dry weight (mg)
The seedlings used for measuring shoot and root lengths were also used for estimating dry weight after removing the cotyledons. These seedlings were dried in hot air oven at 80±1°C for 24 hours. After drying, the mean seedling dry weight was recorded and expressed in milligrams.

Seedling vigour index [SVI]
The seedling vigour indices were calculated by using the formula suggested by Abdul-Baki and Anderson (1973) [1] and expressed in whole number. The formulae are given below.

(i) Seedling vigour index I = Germination (%) x Seedling length (cm)
(ii) Seedling vigour index II = Germination (%) x Seedling dry weight (g)

Speed of germination
During germination test (based on between paper method), daily counts were taken until no further germination was observed (up to 14 days in this case). Data from four replications (100 seeds each) were recorded. An index of the speed of germination was calculated by adding the quotients of the daily counts divided by the number of days of germination (Maguire 1962) [8]. The formula is given below.

$$\text{Speed of germination} = \Sigma\ (n_1/d_1 + n_2\text{-}n_1/d_2 + \ldots\ldots\ldots n_n\text{-}n_n\text{-}1/d_n)$$

Where n= number of seeds germinated on day (d), d= serial number of days

Field emergence (%)
Randomly 100 seeds were selected from each genotype (replicated four times) and sown on a well prepared seed bed at adequate moisture conditions. Seeds are sown uniformly to 4 cm depth with a spacing of 45cm between rows and 15cm between the plants. The number of seedlings emerged at least 4 cm high above the soil surface on 14th day after sowing were counted and expressed in percentage.

Biochemical Traits
Oil content (%)
The oil content (%) was measured on whole seeds (~ 20 g of sample) using nuclear magnetic resonance (NMR) spectroscopy as described by Yadav and Murthy (2016) [12].
Dehydrogenase activity was determined as described by Kittock and Law (1968) using 2, 3, 5-triphenyl tetrazolium chloride solution at 1 per cent concentration. Five seeds from each treatment were taken randomly and preconditioned by soaking in water for 7 h. Seeds were steeped in tetrazolium solution and kept in dark for 3-4 h at 40°C for staining. After staining, the excess solution was drained and the seeds were washed thoroughly with distilled water and transferred to a test tube containing 10 ml of 2-methoxy ethanol (methyl cellosolve). The test tube was closed air tight and allowed to remain in the incubator in darkness overnight for extracting the red coloured formazon. The coloured solution was decanted and the colour intensity was measured in an Optima UV-VIS spectrophotometer using blue filter (470 nm) and methyl cello solve as the blank. The dehydrogenase activity was expressed as OD value or µg per 5 seeds per 10 ml.

Electrical conductivity (EC) of seed leachate (mS/ppt)
Twenty five seeds of two replicates were taken randomly from each treatment in a beaker. Then the seeds were soaked in 50ml of double distilled water for 24 h at 25±10C. The steeped water from soaked seeds was collected and the electrical conductivity (EC) of seed the leachate was measured in a digital conductivity meter (Model: Systronic conductivity meter 306). After subtracting the EC of the distilled water from the value obtained from the seed leachate, the actual EC due to electrolytes was estimated and expressed in mS/ppt.
Total Protein content was determined by the method of Lowry *et al*. (1951) [7] using bovine serum albumin (BSA) as a standard. Extraction of 500 mg of decoated seeds were homogenized using a chilled mortar and pestle in (tissue: buffer ratio 1:2, w/v) ice-cold extraction buffer, pH 8.0, containing 0.1 M phosphate buffer and 1% polyvinylpoly pyrolidine (w/v). The homogenate was centrifuged at 14,000 rpm for 15 min at 4 ◦ C. The resultant supernatant was used for determination of total protein, catalase and peroxidase activity. To a suitably diluted 1 mL extract (prepared as described above) 5.0 mL alkaline copper sulfate was added and mixed well and incubated at room temperature for 10 min. To the resultant solution 0.5 mL Folin Ciocalteau reagent was added and incubated for 30 min and absorbance was read at 660 nm. Concentration of total soluble protein was estimated using a BSA standard curve.
Catalase (CAT) activity was measured following the procedure of Aebi (1984) [2] at 25°C with some minor modifications. The ultra violet (UV) light absorbance of hydrogen peroxide solution can be measured between 230 and 250 nm. On decomposition of hydrogen peroxide by catalase, the absorption decreases with time. Enzyme was extracted as per procedure given above. Three ml of reaction mixture

contained 50 µl enzyme extract, 1.5 ml 100 mM phosphate buffer (pH 7.0), 0.5 ml 75 mM H2O2 and 950 µl distilled water. The control contained enzyme extract and phosphate buffer devoid of H2O2- Catalase activity was estimated by the decrease in absorbance of H2O2 at 240 nm and expressed as µmol H2O2 decomposed/min/g.

Peroxidase (POX) activity was assayed, as increase in optical density due to oxidation of guaiacol to tetra-guaiacol following Castillo *et. al*. (1994) with minor modifications at 470 nm absorbance using a reaction mixture containing 12 mM hydrogen peroxide and 96 mM guaiacol in phosphate buffer (pH 7.0). Three ml reaction mixture contained one ml 100 mM phosphate buffer (pH 7.0); 0.5 ml each 96 mM guaiacol; 12 mm H2O2; 50 µl enzyme extract and 950 µl distilled water. Absorbance due to the formation of tetra-guaiacol was recorded at 470 nm and enzyme activity was calculated as per the extinction coefficient of its oxidation product, tetra–guaiacol E =26.6 nM/cm. Enzyme activity was expressed as µmoles/cm/min/g seed fresh weight.

Statistical analysis

Simple correlation analysis based on Pearson correlation coefficients (*r*) was performed using 'Data Analysis option implemented in MS Excel.

Results and Discussion

Seed breadth exhibited strong positive association with length breadth thickness product (r=0.975**) and had strong negative association with length breadth ratio (r=-0.942*). The results suggested that seed breadth contributes for larger seed size. Seed thickness showed strong positive correlation with length breadth thickness product (0.920*), germination (0.936*) and field emergence (0.933*) and strong negative correlation with bulk density (-0.971**). The results suggested that increase in seed thickness can lead to larger seed size, higher germination and better field emergence. However, it appeared that increase in seed thickness may reduce bulk density. Length breadth ratio showed strong negative association with seed breadth (*r*=-0.942**) and length breadth thickness product (*r*=-0.893*). These correlations are expected as length breadth ratio is a derived parameter from seed breadth. Length breadth thickness product showed strong positive correlation with seed breadth (*r*=0.975**) and seed thickness (*r*=0.920**). It showed strong negative correlation with length breadth ratio (*r*=-0.893*) and bulk density (*r*=-0.878*). Length breadth thickness product is a derived parameter from seed breadth and seed thickness; therefore, linear correlations were observed. In contrast, length breadth thickness product showed strong positive correlations with all the seed size and weight parameters like seed breadth, seed thickness and bulk density. Therefore, it appears that length breadth thickness product is more informative than length breadth ratio in revealing relationship among seed size and weight parameters. Test weight showed strong positive correlation was observed with Hull content (0.919). These relationships suggest that higher seed weight would result in increase of hull content. Bulk density was observed weak negative correlation with germination (-0.877*) and field emergence (-0.885*).

Germination recorded weak positive correlation with Speed of ger (0.877*) and strong correlation with FE (0.984**). Naderidar baghshahi (2013) [10] also showed better efficiency of standard germination in prediction of field emergence in bean, cotton, barley, sunflower. Speed of germination showed strong positive correlation with field emergence (0.940**). The results suggested that seeds with more speed of germination have better field emergence. Seedling Length was observed weak positive correlation with Seedling vigour index 1 and seedling vigour index 2 (0.882*). A significant negative correlation (-0.887*) was determined between seed germination and protein content in seed. Marinkovic *et al*. (2003) [9] concluded that in addition to the study of physiological aspect of protein synthesis in sunflower, attention should be devoted to the study of genetic and individual environmental factors that increase the protein content in seed. Dehydrogenase activity has strong positive correlation was observed with test weight (0.948**) and weak positive correlation with dry weight (0.889*) respectively. The variation germination among genotypes may be mainly due to oxygen uptake and dehydrogenase activity which may influence the germinative capacity in two ways: respiratory activity provides energy to the germinating embryo and respiratory activity reflects both the integrity and overall capacity of the metabolic machinery, which was in support of finding of Woodstock (1973) [11].

Table 1: Simple correlation coefficients among seed traits in sunflower hybrids

	OC %	PC (mg/g)	CAT	PER	DH	EC	SL	SB	ST	LBR	LBTP
OC %	1										
PC (mg/g)	0.382	1									
CAT	-0.549	-0.615	1								
PER	0.199	-0.026	0.556	1							
DH	-0.825	-0.623	0.299	-0.572	1						
EC	0.042	0.822	-0.104	0.387	-0.547	1					
SL	0.769	-0.055	0.110	0.628	-0.719	-0.085	1				
SB	0.349	-0.633	0.402	0.311	-0.095	-0.643	0.757	1			
ST	0.298	-0.763	0.193	0.117	0.096	-0.837	0.542	0.848	1		
LBR	-0.083	0.805	-0.459	-0.074	-0.240	0.816	-0.494	-0.942*	-0.849	1	
LBTP	0.448	-0.621	0.292	0.328	-0.158	-0.660	0.788	0.975**	0.920*	-0.893*	1
TW	-0.732	-0.441	0.011	-0.721	0.948*	-0.475	-0.833	-0.306	-0.001	-0.021	-0.321
BD	-0.462	0.612	-0.003	-0.121	0.059	0.752	-0.585	-0.764	-0.971**	0.716	-0.878*
HC (%)	-0.410	-0.427	-0.261	-0.811	0.804	-0.645	-0.649	-0.171	0.226	-0.112	-0.127
SDL (cm)	-0.345	-0.344	0.581	0.671	0.043	0.155	0.006	-0.036	0.127	0.076	0.038
G (%)	0.017	-0.887*	0.419	0.229	0.260	-0.778	0.372	0.734	0.936*	-0.776	0.805
Dry.Wt (g)	-0.802	-0.813	0.676	-0.209	0.889*	-0.572	-0.405	0.242	0.269	-0.522	0.138
SVI-I	-0.227	-0.695	0.611	0.562	0.171	-0.305	0.195	0.362	0.570	-0.360	0.445
SVI-II	-0.227	-0.695	0.611	0.562	0.171	-0.305	0.195	0.362	0.570	-0.360	0.445
SG	0.101	-0.722	0.639	0.653	-0.061	-0.440	0.620	0.780	0.789	-0.704	0.820

FE	0.123	-0.823	0.449	0.379	0.101	-0.686	0.515	0.788	0.933*	-0.774	0.860

Correlation coefficients at 1 % and 5 % level of significance are 0.958 and 0.878, respectively for n=5

OC- Oil Content, PC- Protein Content, CAT- Catalase Activity, POD- Peroxidase activity, ,DH - Dehydrogenase Activity, EC- Electrical Conductivity, SL- Seed Length, SB- Seed Breadth, ST- Seed Thickness, LBR- Length Breadth Ratio, LBTP- Length Breadth Thickness Product, HC- Hull Content, TW- Test Weight, BD- Bulk Density, HC – Hull Content, G- Germination, SG- Speed of Germination, SDL- Seedling Length, SDW- Seedling Dry Weight, SVI-I -Seedling Vigour Index-I, SVI-II- Seedling vigour Index-II, FE- Field Emergence

Table (Cond...,)

	T W (g)	**BD(kg/m3)**	**H C (%)**	**S. len (cm)**	**Ger(%)**	**Dry.Wt (g)**	**SVI 1**	**SVI 2**	**Speed of Ger**	**F E**
BD (kg/m3)	0.091	1								
HC (%)	0.919*	-0.198	1							
S .Len (cm)	-0.022	-0.113	-0.168	1						
Ger(%)	0.138	-0.877*	0.264	0.427	1					
Dry.Wt (g)	0.701	-0.057	0.502	0.207	0.456	1				
SVI 1	0.061	-0.527	0.026	0.882*	0.803	0.385	1			
SVI 2	0.061	-0.527	0.026	0.882*	0.803	0.385	0.812	1		
Speed of Ger	-0.255	-0.725	-0.215	0.594	0.877*	0.296	0.850	0.850	1	
FE	-0.037	-0.885*	0.091	0.461	0.984**	0.351	0.817	0.817	0.940*	1

Correlation coefficients at 1% and 5% level of significance are 0.958 and 0.878, respectively for n=5

OC- Oil Content, PC- Protein Content, CAT- Catalase Activity, POD- Peroxidase activity, ,DH - Dehydrogenase Activity, EC- Electrical Conductivity, SL- Seed Length, SB- Seed Breadth, ST- Seed Thickness, LBR- Length Breadth Ratio, LBTP- Length Breadth Thickness Product, HC- Hull Content, TW- Test Weight, BD- Bulk Density, HC – Hull Content, G- Germination, SG- Speed of Germination, SDL- Seedling Length, SDW- Seedling Dry Weight, SVI-I -Seedling Vigour Index-I, SVI-II- Seedling vigour Index-II, FE- Field Emergence

Conclusions

Overall, physical seed traits relationships suggest that germination, field emergence and speed of germination content can be improved by seed size (seed thickness, length breadth thickness ratio) traits and may be decreased through increase in bulk density. Reducing the protein content without affecting the other traits can able to increase the germination. As test weight is related to dehydrogenase activity, indirectly it plays a role in germination, dry weight, seedling vigour indexes as well as hull content. The correlations among traits may occur due to linkage or pleiotrophy and are important for plant breeders to make selection decisions. Therefore, it is essential that biological basis of such correlations need to be understood. With advancements in genetics and genomics research, it has become possible to explain such trait relations at molecular level using either germplasm or pedigree based populations.

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