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Association of Growth Habit and Anthocyanin Pigment with Winter Hardiness in Lentil*

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Abstract
To determine association between any two of the three traits of cold tolerance, growth habit and anthocyanin pigment in lentil (*Lens culinaris* Medikus), F_2 populations were studied for two years (1991/92 and 1992/93) at Quetta and F_3 for one year (1992/93) at two different field sites, Quetta and Kalat, Balochistan, Pakistan. Highly significant ($p<0.001$) association between any two of the three traits in all possible combinations was found which indicates that indirect selection for winter hardiness, using prostrate growth habit and anthocyanin pigment, is possible.

Introduction
Approximately 50 percent of the area of the Balochistan province of Pakistan is described as arid uplands (≥1000 m) and is representative of the arid highlands of W. Asia (Ali *et al*., 1991). Highly variable and unpredictable environmental conditions (primarily cold and drought) prevail in the region where lentil is cultivated. With the improvement of winter hardiness acreage of lentil may be increased in the region which may lead to increase in the total production.

Direct selection for winter hardiness is the best approach for improvement of this trait in lentil but under natural conditions it is not possible without having differential winter kill in the field which can only be seen in certain number of years when the air temperatures are less than or equal to -15°C (personal observation). Furthermore, the natural environments are not reliable and controllable due to their various inherited problems. To speed up the breeding work and to improve its efficiency, it is essential to find an association among morphological traits for winter hardiness to make indirect selection for this trait in the breeding material of lentil when extreme low air temperatures in the field are not experienced.

There are few studies in other crop species indicate association among morphological traits for winter hardiness. The development of a compact rosette of shoots was found essential for winter survival in pea (Markarian and Andersen, 1966; Andersen and Markarian, 1968). Growth habit and cold tolerance have been found to be related in barley. The more upright types were injured more by freezing temperatures when compared to prostrate types under field conditions (Abo-Elenein *et al*., 1967). Reid (1965) established a definite association between winter growth habit and high winter survival but this association was not complete and hardy spring lines of barley were also isolated. Pea plants with high anthocyanin (purple pigment) have the greater number of cold hardy segregates (Markarian *et al*., 1968). However, the authors were not sure that the anthocyanin content itself was responsible for increased hardiness or if it was linked or associated with the factors responsible for winter hardiness. Ellis and Hong (1995) found that lentil genotypes which survive cold winters also have a lower cool base temperatures for germination, but their findings revealed that it is not possible to select cold tolerant genotypes directly from estimates of the base temperature for germination. The objective of this study was to determine association between any two of the three traits of cold tolerance, growth habit and anthocyanin pigment in lentil.

Materials and Methods
Meteorological data were recorded in each year and at each of two field sites, Quetta (altitude 1750 m, latitude 30° 14' N, longitude 67° 2' E) and Kalat (altitude 1850 m, latitude 29° 07' N, longitude 66° 24' E), in Balochistan province of Pakistan. Above average rainfall (280 and 308 mm respectively) was received at Quetta in both growth seasons of 1991/92 and 1992/93. The minimum temperature in both seasons was -7°C. Although temperatures never fell below zero during January of the 1991/92 season but January, 1993 had 14 days below at Quetta. There were quite few genotypes injured during 1991/92. The low temperatures at the Kalat location were similar to Quetta. Substantially reduced rainfall (39 mm) during the growth period was received at Kalat.

Five different lines and/or cultivars, including winter hardy and spring lentils, were selected for crossing. All winter hardy lines had prostrate growth habit and anthocyanin pigment present whereas spring lentil had erect growth habit and pigment absent. Three males Balochistan Local, ILL 5865 (recently released variety in Pakistan as SHIRAZ-96) and ILL 5677 (all winter hardy) and two females, WA 8649090 (WA) (winter hardy) and Brewer (non-hardy spring lentil), were used in six one-way crosses following a factorial design (North Carolina Design-II) format (Comstock and Robinson, 1948). Two sets of F, seed were made. One set (F2 population) was planted at Quetta on October 19, 1991 and the second set on October 13, 1992 at the same site. At least six seeds were sampled from each F2 plant, bulked across all F2 plants within each cross and divided into two sets of two replications each to grow F_3 bulk populations during the next season at Quetta and at Kalat. The experiments during the 1992/93 season were planted on October 12, 1992. In all experiments, all crosses were nested with their parents in families in two replications in the field. Each plot was space-planted with 140 seeds to obtain at least 128 plants per plot in a randomized complete block design (RCBD). The plant-to-plant and row-to-row distance was 50 cm. A pre-planting irrigation of c. 50 mm was applied to all plots to ensure emergence. The experiments were then dependent upon rainfall until, plant maturity. Soil at both sites was a sandy clay loam. Nitrogen and phosphate fertilizers at 20 and 60 kg ha^{-1} respectively were incorporated into the soil at the time of seedbed preparation. Cold tolerance was scored on a scale of 1-5 where 1 = no foliar damage and 5 = completely dead. It was recorded on March 27 when clear symptoms of cold injury after possible recovery of the plants could be seen (Auld *et al*., 1983; Ali *et al*., 1991). On 15th of December, when the plants were fully acclimatized, growth habit data were recorded on the scale of 1-3 where 1 was used for prostrate, 2 for semi-prostrate and 3 for erect growth habit. At the same time, pigment data were recorded as 1 = presence of pigment and 2 = absent.

A Statistical Analysis System (SAS) computer software program was used to analyse the data. Chi-square test of independence was used to determine the association between any two of the three traits of cold tolerance, growth habit and anthocyanin pigment (SAS, 1988).

Results

An extremely large number of genotypes having no cold injury also had prostrate growth habit and anthocyanin pigment present consistently in all plant populations in two years of field study across the locations and generations (Table 1-4). Using this data, chi-squared test of independence showed that all three traits (cold tolerance, growth habit and pigment) in all possible combinations in all experiments at all sites were highly significantly ($p < 0.001$) associated (Table 5).

Table 1: Number of plants falling in each possible group in all possible combinations among the traits of cold tolerance, growth habit and anthocyanin pigment in F_2 population at Quetta during 1991/92 crop season

a) Growth habit by cold tolerance

Growth habit	Cold injury scores					
	1	2	3	4	5	Total
1	1531	0	0	0	0	1531
2	251	0	0	0	0	251
3	545	4	4	2	3	558
Total	2327	4	4	2	3	2340

b) Growth habit by pigment

Growth habit	Pigment		
	1	2	Total
1	1525	6	1531
2	4	247	251
3	1	557	558
Total	1530	810	2340

c) Pigment by cold tolerance

Pigment	Cold injury scores					
	1	2	3	4	5	Total
1	1530	0	0	0	0	1530
2	797	4	4	2	3	810
Total	2327	4	4	2	3	2340

Table 2: Number of plants falling in each possible group in all possible combinations among the traits of cold tolerance, growth habit and anthocyanin pigment in F_2 population at Quetta during 1992/93 crop season

a) Growth habit by cold tolerance

Growth habit	Cold injury scores					
	1	2	3	4	5	Total
1	714	11	3	0	1	729
2	75	74	46	21	65	281
3	25	85	44	14	115	283
Total	814	170	93	35	181	1293

b) Growth habit by pigment

Growth habit	Pigment		
	1	2	Total
1	718	11	729
2	155	126	281
3	79	204	283
Total	952	341	1293

c) Pigment by cold tolerance

Pigment	Cold injury scores					
	1	2	3	4	5	Total
1	775	91	34	13	39	951
2	39	79	59	22	142	341
Total	814	170	93	35	181	1293

Discussion

Natural environments are highly inconsistent, unpredictable and non-repeatable. Moreover, confounding effected different factors like diseases, insect damage, frost heavy planting date and various other cultural practices encountered in the field make field selection for hardiness difficult and unreliable. Years/locations, such as in 1991/92 at Quetta, can make direct selection impossible and ultimately progress would be slow as the expression of cold tolerance is extremely dependent upon the severity of the environment. Indirect selection is a likely alternative in this kind of situation. A significant ($p<0.001$) association among cold tolerance, growth habit and pigmentation is evident, which may make selection for winter hardiness

Table 3: Number of plants falling in each possible group in all possible combinations among the traits of cold tolerance, growth habit and anthocyanin pigment in F_3 population at Quetta during 1992/93 crop season

a) Growth habit by cold tolerance

Growth habit	Cold injury scores					
	1	2	3	4	5	Total
	772	27	1	2	1	803
2	236	116	30	14	14	410
3	142	148	53	19	109	471
Total	1150	291	84	35	124	1684

b) Growth habit by pigment

Growth habit	Pigment		
	1	2	Total
1	772	31	803
2	280	130	410
3	119	352	471
Total	1171	513	1684

c) Pigment by cold tolerance

Pigment	Cold injury scores					
	1	2	3	4	5	Total
1	973	131	31	21	15	1171
2	777	160	53	14	109	513
Total	1150	291	84	35	124	1684

Table 4: Number of plants falling in each possible group in all possible combinations among the traits of cold tolerance, growth habit and anthocyanin pigment in F_3 population at Kalat during 1992/93 crop season

a) Growth habit by cold tolerance

Growth habit	Cold injury scores					
	1	2	3	4	5	Total
1	717	0	0	0	0	717
2	332	8	0	0	0	340
3	154	182	48	10	15	409
Total	1203	190	48	10	15	1466

b) Growth habit by pigment

Growth habit	Pigment		
	1	2	Total
1	485	232	717
2	144	196	340
3	30	379	409
Total	659	807	1466

c) Pigment by cold tolerance

Pigment	Cold injury scores					
	1	2	3	4	5	Total
1	654	5	0	0	0	659
2	549	185	48	10	15	807
Total	1203	190	48	10	15	1466

Table 5: Chi-squared values for the test of independence of traits of growth habit (Gh), cold tolerance (Ct) and pigment (Pm) in all possible combinations in all populations, years and locations. All values are highly significant ($p<0.001$), n = sample size

Traits	Generation, year and location			
	F_2 1991/92 Quetta	F_2 1992/93 Quetta	F_3 1992/93 Quetta	$F_3$1992/93 Quetta
Gh/Ct^H	41.75	921.56	701.54	761.47
Pm/Ct	24.69	573.25	442.77	240.20
Gh/Pm	2291.79	586.00	704.36	384.00

possible under field conditions when differential winter kill is not available. Large numbers of plants having prostrate growth habit and pigment were not injured at all in the field during 1992/93 at both sites when damage to other plants in response to low temperatures was observed. This indicates that indirect selection for winter hardiness can be made by using prostrate growth habit and purple pigmentation.

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