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# IDENTIFICATION OF ELITE PURE-LINES FROM LOCAL LENTIL GERMPLASM USING DIVERSITY INDEX BASED ON QUANTITATIVE TRAITS

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**Abstract**

Three hundred and seventeen accessions of lentil collected from all over the country were evaluated for six quantitative traits to investigate inter and intra-accession diversity in association with geographic pattern. Variation indicated that areas with high geographic diversity that is supposed to present high biological diversity are yet to be explored. Classification of germplasm gave rise to some elite lines for specific characters and the accessions for days to flowering (45), days to maturity (7), plant height (12), pods per cluster (17) and seed weight (27) have been selected and suggested for exploitation in breeding programme. Twelve clusters were observed with varying degrees of inter-cluster dissimilarity that suggested the selection of diverse superior parents for hybridization. Some of the characters associated with origin as high seed weight of germplasm collected from Baluchistan is needed to exploit for specific trait/s. Germplasm distribution revealed that Punjab and Sindh represented a high collections along with high diversity, whereas other areas are yet to be explored. Similarly zone 3a, 6, 7, 9 and 10 along with high mountains lack complete representation that indicated the importance for future collection mission to these areas.

## Introduction

Plant breeding, the induced evaluation changed the phyto history in the recent past and the improvement in crop plants is mainly based on the presence of genetic variation either natural or induced through gene recombinant, mutation or transformation (Ghafoor *et al*., 1989). Local germplasm of crop plants is an excellent source of economically useful plant characters (Pecetti *et al*., 1996). The breeders must have a mean of choosing the accession most likely to posses the trait of interest. Quantitative traits provide an estimate of genetic diversity and various numerical taxonomic techniques have been successfully used to classify and measure the pattern of phenotypic diversity in the relationship of germplasm collections in a variety of crops by many scientists as in lentil (Ahmed *et al*., 1997, Fratini *et al*., 2007 and Tullu *et al*., 2008), Pea (Amurrio *et al*., 1995) and Alfalfa (Smith *et al*., 1991, 1995 and Warburton & Smith, 1993). Rubeena *et al*., (2006) identified markers closely linked to the major QTL that may be useful for future marker-assisted selection for crop improvements. Lentils are an old world legume and were probably one of the first plant species to be domesticated (Bahl *et al*., 1993). Nutritionist rank lentil as an excellent source of diet which is high in protein, a major source of complex carbohydrates, high in fibers, rich in vitamins A and B, potassium and iron, low in sodium and fat that regulate growth and development (Anon., 2003).

Worldwide, lentil is grown on a total of 1.8 million hectares, of which 60% is in the South Asian region which includes the lentil producing countries of Bangladesh, Burma, India, Nepal and Pakistan (Nazir *et al*., 1994). In Pakistan, lentil is annually grown as a winter-season (rabi) as second important pulse with an area of 44800 ha and 26200 tones production next to chickpea under rainfed areas (Anon., 2002). In spite of its dietary significance and important role it plays in the farming system, researchers allocate low priority to this valuable commodity. Consequently lentil remains the least researched and can be proclaimed the least understood of the cultivated food legumes.

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Therefore, to enhance the scope and usefulness of genetic resources of lentil germplasm, present study was under taken to access and evaluate the genetic diversity in lentil germplasm collected from all over the country on the basis of quantitative traits and to identify superior genotypes for future use.

## Materials and Methods

Three hundred and seventeen accessions of lentil collected from all over the country and preserved in the gene bank of Plant Genetic Resources Programme (PGRP), National Agricultural Research Center (NARC), Islamabad were included in present study (Bhatti *et al*., 1997). Of these, 199 accessions were collected from Punjab, 57 from Sindh, 48 from Baluchistan, 4 from NWFP, 4 from Northern Areas (NA) and 5 were unknown. The experiment was conducted under field and green-house conditions at Plant Genetic Resources Programme, National Agricultural Research Center, Islamabad (33.40$^o$N and 73.07$^o$E). Two checks, Masoor 85 and Manshera 89 were included in this study after every 10 rows. One row for each accession was planted with 30 and 5 cm inter and intra-row spacing, respectively. Recommended cultural practices were followed throughout the crop season.

Ten plants were sampled at random to study inter and intra-accession variation. Quantitative traits (days to flowering, days to maturity, plant height, pods per cluster, seeds per pod and 100-seed weight) were recorded on 10 plants following IBPGR Descriptors (Anon., 1985). The data were averaged for simple statistics (mean, standard deviation, coefficient of variation), and simple correlation coefficients using computer software "Microsoft Excel" for Window following the methods of Steel & Torrie (1981). Data were also analyzed for numerical taxonomy using the procedure of Sneath & Sokal (1973).

## Results and Discussion

Basic statistics for quantitative traits revealed high variance for all the characters except for pods per cluster, seed per pod and seed weight where a low variance was observed (Table 1). As this study was conducted on a high number of local germplasm accessions that displayed high range and variance that enhanced the acceptance of result. Present study revealed sufficient variability in lentil germplasm to allow selection for the characters studied. Variability for these traits in lentil germplasm was also reported by Agrawal *et al*., (1976), Tiwari & Singh (1980), Malik *et al*., (1984) and Toklu *et al*., (2009). Singh & Singh (1993) confirmed the wide range of variation in agronomic characteristics of lentil germplasm, except for seeds per pod. Wild *Lens* species/ subspecies are a potential source for increasing genetic diversity in cultivated lentil (Gupta & Sharma, 2007).

**Table 1. Basic statistics for quantitative characters in lentil germplasm.**

| Traits | When and where measured | Mean ± SE | σ | σ² % | Range |
|---|---|---|---|---|---|
| Days to flowering | At 50% flowering | 119 ± 0.79 | 14.15 | 168.2 | 88 – 151 |
| Days to flowering | At 90% maturity | 172 ± 0.49 | 8.78 | 44.8 | 100 –215. |
| Plant height | At advance pod-filling stage, measured form ground to the tip of extended foliage | 45.12 ± 0.46 | 8.25 | 153.1 | 24.00 –70.00 |
| Pods per cluster | At the time of maturity just before harvest | 2.02 ± 0.03 | 0.60 | 17.3 | 0.70 –3.20 |
| Seeds per pod | After harvesting | 1.82 ± 0.01 | 0.26 | 3.8 | 0.70 –2.00 |
| Seed weight | After harvesting | 1.90 ± 0.03 | 0.54 | 15.3 | 0.74 –5.22 |

SE-Standard Error, σ- Standard Deviation and σ$^2$ %-Variance Percentage

Subdividing variance into its components assists genetic resources conservation, utilization and it enables planning for use of appropriate gene pools in crop improvement for specific plant attributes (Bekele, 1984 & 1985). As a low variance was observed for pods per cluster, seed per pod and seed weight and this finding is in conformity with the findings of Balyan & Singh (1986b); hence, the genes for these important economic traits should be investigated/ exploited from other source. Evaluation of germplasm under greenhouse and field conditions indicated a linear relationship for days to flowering, maturity and plant height; hence the data recorded under field conditions were presented. Large scale testing of broad base germplasm needs to be built up by making extensive local collection and obtaining germplasm from abroad to develop a sound breeding program (Jain *et al*., 1975; Ghafoor *et al*., 1992). Brown (1978), Laghetti *et al*., (1998) and Gupta & Sharma (2007) advocated that maximum genetic conservation would be achieved by sampling population from as many environments as possible to widen the genetic base of the cultivated lentil.

Classification of germplasm gave rise to some elite lines for specific characters and the accessions for days to flowering (45), days to maturity (7), plant height (12), pods per cluster (17) and seed weight (27) have been selected and suggested for exploitation in breeding program (Table 2). Seven accessions (66097, 66036, 66014, 66015, 66104, 66022, 66002) matured in less than 160 days after planting and were earlier than Mansehra 89. Short duration is one of the important characters in legumes as described by Bakhsh *et al*., (1992) and should be utilized for the development of short duration lentil cultivar. Eight accessions (66004, 66029, 66302, 66303, 66304, 66314, 66316, 66317) gave seed weight more than 3.1 g and hence could be utilized for the manipulation of this trait as high seed weight in any grain crop is preferred by the consumers.

It was observed that some of these accessions possessed desirable genes for more than one character and hence these could be utilized directly or included in hybrid programme for varietal development. Selected accessions are suggested to be tested under a wide range of agro-ecological conditions for their potential confirmation and if found better under diversified and/or specific environments, should be exploited in lentil selection/breeding program. Phenogram of twelve clusters was constructed (Fig. 1) and the dissimilarity coefficient ranged from 0.46 (Cluster I Vs IV) to 2.85 (Cluster V Vs X) among all the accessions (Table 3). The inter-cluster distance among the accessions revealed that the cluster V consisting of five accessions was obviously very much different from all the other clusters with a genetic distance ranging from 1.99 (Cluster VIII) to 2.85 (Cluster X). Exploring the germplasm of 12 clusters for distribution based on geographical parameters revealed the presence of cluster V with important features. In case of cluster V, all the accessions belonged to Baluchistan and were the best for seed weight but late in maturity. Germplasm was grouped into twelve clusters based on average linkage.

Details of each cluster including mean and standard deviation for each cluster for quantitative characters presented in Table 4 revealed that cluster I consisted of 30 accessions, cluster II representing 28 accessions, cluster III of 45 accessions, cluster IV of 36 accessions, cluster V of 5 accessions, cluster VI of 19 accessions, cluster VII of 14 accessions, cluster VIII of 20 accessions, cluster IX representing 51 accessions, cluster X representing of 31, cluster XI 21 and cluster XII representing of 19 accessions. Cluster I and X were observed with maximum seeds per pod. Cluster II was observed with maximum plant height. Cluster IV was observed with maximum pods per cluster. Cluster V was best for seed weight but late in flowering and maturity. Cluster IX was early in flowering and maturity. Figure 2 indicated the frequency of germplasm distribution on the basis of three geographic parameters. Punjab and Sindh represented high collections along with high diversity, whereas others are yet to be explored for maximum genetic diversity. Similarly zone 3a, 6, 7, 9 and 10 along with high mountains lack complete representation that indicated the importance for future collection mission to these areas.

**Table 2.The selected accessions on the basis of best performance for specific characters.**

| Character | range | |
|---|---|---|
| Days to flowering | < 100 days | M-85, M-89, 66002, 66007, 66010, 66014, 66015, 66018, 66022, 66023, 66024, 66027, 66029, 66031, 66032, 66036, 66039, 66041, 66064, 66072, 66074, 66079, 66082, 66083, 66084, 66091, 66094, 66095, 66097, 66098, 66099, 66101, 66104, 66106, 66108, 66110, 66112, 66125, 66126, 66136, 66168, 66229, 66231, 99284, 66289, 66297 |
| Days to maturity | < 160 days | M-85, M-89, 66002, 66014, 66015, 66022, 66036, 66097, 66104 |
| Plant height | > 60 cm | 66006 ,66038, 66050, 66053, 66057, 66059, 66076, 66119, 66130, 66132, 66160, 66227, 66300, 66302, 66304, 66309 |
| Pods per cluster | > 3 clusters | 66045, 66047, 66100,66121, 66176,66179, 66201, 66212, 66214, 66216, 66217, 66218, 66225, 66239, 66242,66282, 66293 |
| Seed weight | > 2.5 g | M-89, 66004, 66010, 66027, 66029, 66030, 66031, 66039, 66042, 66074, 66113, 66136, 66183, 66185, 66187, 66190, 66192, 66284, 66293, 66297, 66302, 66303, 66304, 66314, 66316, 66317, 66323, 99229 |

**Table 3. Inter cluster distance in twelve clusters based on qualitative traits lentil germplasm.**

| | C-I | C-II | C-III | C-IV | C-V | C-VI | C-VII | C-VIII | C-IX | C-X | C-XI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C –II | 0.95 | | | | | | | | | | |
| C –III | 0.58 | 0.83 | | | | | | | | | |
| C –IV | 0.46 | 0.61 | 0.73 | | | | | | | | |
| C –V | 2.79 | 2.55 | 2.74 | 2.76 | | | | | | | |
| C –VI | 0.93 | 1.01 | 0.61 | 1.01 | 2.72 | | | | | | |
| C –VII | 0.98 | 1.45 | 0.91 | 1.29 | 2.34 | 0.92 | | | | | |
| C –VIII | 1.35 | 1.30 | 1.21 | 1.42 | 1.99 | 1.56 | 1.23 | | | | |
| C –IX | 1.28 | 1.57 | 0.94 | 1.51 | 2.79 | 0.66 | 0.86 | 1.72 | | | |
| C –X | 0.50 | 1.19 | 0.67 | 0.83 | 2.85 | 1.20 | 1.04 | 1.14 | 1.39 | | |
| C –XI | 0.95 | 0.95 | 0.93 | 0.95 | 2.44 | 1.33 | 1.35 | 0.83 | 1.62 | 0.84 | |
| C –XII | 1.69 | 1.59 | 1.52 | 1.74 | 2.15 | 1.72 | 1.66 | 1.04 | 1.80 | 1.56 | 0.88 |

C- Cluster, The values in the parenthesis is number of accessions in a particular cluster, C-I (30), C-II (28), C-III (45), C-IV (36), C-V (5), C-VI (19), C-VII (14), C-VIII (20), C-IX (51), C-X (31), C-XI (21), C-XII (19)

**Table 4. Means and standard deviation for quantitative characters for 12 clusters in lentil germplasm.**

| Clusters | Days to flowering | Days to maturity | Pods per cluster | Seeds per pod | Plant height (cm) | Seed weight (g) |
|---|---|---|---|---|---|---|
| Cluster I | 115 ± 6.84 | 170 ± 3.53 | 2.67 ± 0.26 | 1.96 ± 0.07 | 44.67 ± 3.28 | 1.70 ± 0.19 |
| Cluster II | 126 ± 6.38 | 176 ± 4.08 | 2.09 ± 0.27 | 1.87 ± 0.17 | 59.89 ± 4.55 | 1.71 ± 0.31 |
| Cluster III | 118 ± 4.63 | 170 ± 3.91 | 1.84 ± 0.21 | 1.93 ± 0.10 | 45.13 ± 4.70 | 1.64 ± 0.16 |
| Cluster IV | 119 ± 4.80 | 171 ± 2.35 | 2.71 ± 0.21 | 1.94 ± 0.09 | 53.39 ± 3.74 | 1.62 ± 0.11 |
| Cluster V | 142 ± 4.16 | 190 ± 0.89 | 1.94 ± 0.18 | 1.40 ± 0.49 | 52.60 ± 11.9 | 4.73 ± 0.30 |
| Cluster VI | 102 ± 4.48 | 168 ± 3.00 | 1.54 ± 0.29 | 1.85 ± 0.16 | 49.63 ± 5.17 | 1.91 ± 0.32 |
| Cluster VII | 107 ± 7.12 | 174 ± 6.14 | 1.90 ± 0.31 | 1.95 ± 0.11 | 36.79 ± 4.02 | 2.60 ± 0.29 |
| Cluster VIII | 142 ± 3.03 | 186 ± 6.96 | 1.90 ± 0.50 | 1.78 ± 0.20 | 40.15 ± 5.49 | 2.36 ± 0.40 |
| Cluster IX | 100 ± 2.58 | 163 ± 10.80 | 1.17 ± 0.25 | 1.79 ± 0.24 | 39.69 ± 4.79 | 2.17 ± 0.43 |
| Cluster X | 125 ± 3.67 | 173 ± 2.01 | 2.54 ± 0.31 | 1.96 ± 0.08 | 37.26 ± 4.30 | 1.55 ± 0.18 |
| Cluster XI | 134 ± 8.71 | 180 ± 5.13 | 2.37 ± 0.37 | 1.58 ± 0.16 | 44.86 ± 5.01 | 1.64 ± 0.29 |
| Cluster XII | 138 ± 9.85 | 182 ± 5.60 | 1.78 ± 0.44 | 1.15 ± 0.20 | 40.89 ± 6.67 | 2.08 ± 0.28 |

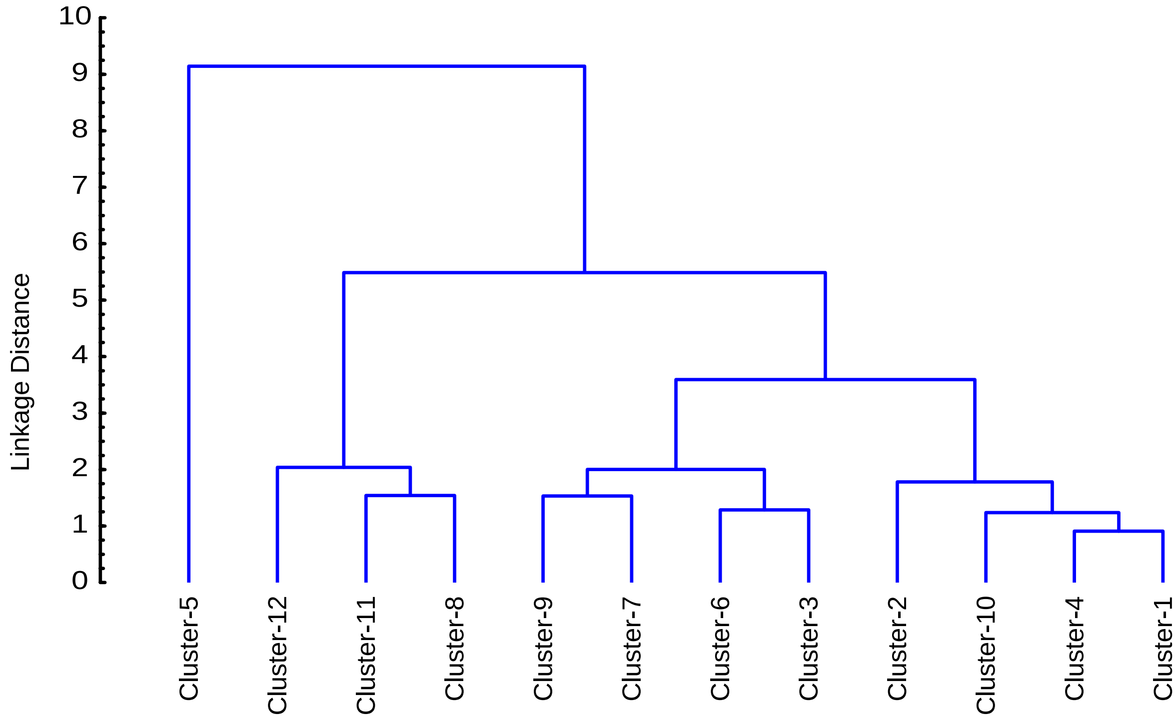


Fig. 1. Phenogram of twelve clusters based on average linkage distance in lentil germplasm.

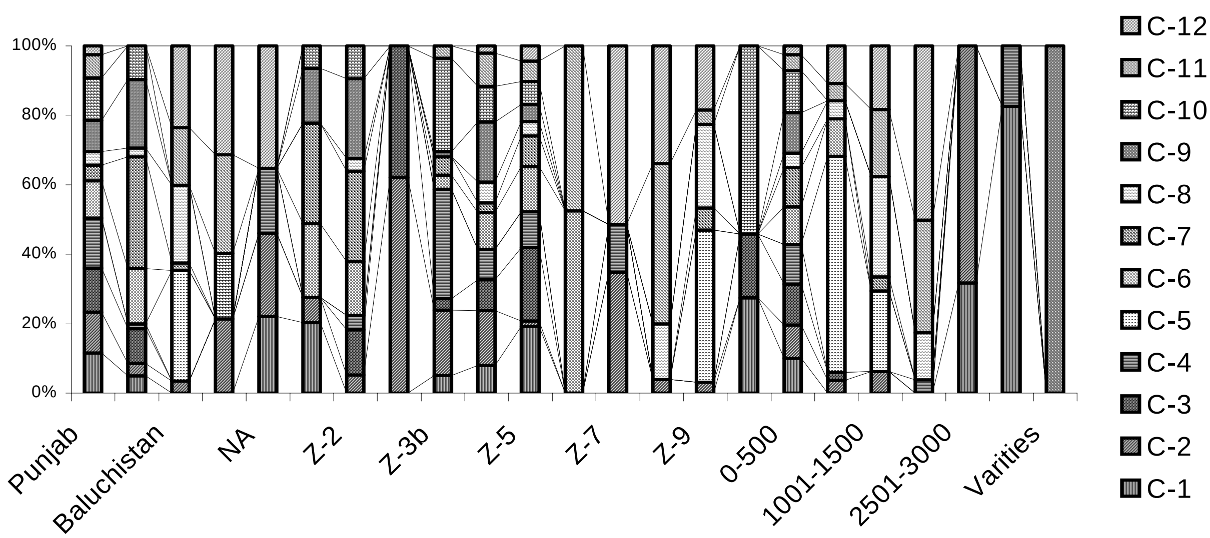


Fig. 2. Inter-relationship of genetic diversity based on quantitative traits and three geographic parameters (Provinces, Agro-ecological zones and Altitude) in lentil germplasm.

In the material used, correlation was conducted for each province separately along with pool data (Table 5). Indifferent results for correlation in various character pairs indicated that the germplasm collected from different regions is needed to use independently for selecting superior pure-lines from each set of cluster. Although pods per cluster vs seeds per pod were positively associated in same direction and magnitude. Correlation coefficient measures the degree to which a variable varies together or a measure of the intensity of association. It further confirms the interrelationship of the metric traits, which are essential for designing breeding strategy (Islam *et al*., 1990 and

Toetia *et al*., 1983). Thus, knowledge of interrelationship among these characters is very critical. In general, correlation results revealed that selection within different clusters could be practiced for different traits and suitable parents could be selected for further development. Peyghambary (2003) reported similar correlation between flowering and maturity and negative correlation between seeds per pod and seed weight. Findings of the present study are in consistent with Amurrio *et al*., (1993) who reported positive correlation between days to flowering and days to maturity. In the present study negative correlation was observed between time to flowering and seed weight. These findings are in conformity with Bhatt (1977), Sarwar *et al*., (1982), Balyan & Singh (1986a) and Bakhsh *et al*., (1991).

**Table 5. Simple correlation among six quantitative traits for provinces in lentil germplasm.**

| Quantitative traits | Provinces | Days to flowering | Days to maturity | Pods per cluster | Seeds per pod | Plant height |
|---|---|---|---|---|---|---|
| Days to maturity | Baluchistan | 0.11** | | | | |
| | NA | -0.17** | | | | |
| | NWFP | -0.65** | | | | |
| | Punjab | 0.22** | | | | |
| | Sindh | 0.67** | | | | |
| | Un known | 0.14* | | | | |
| | Pooled | 0.80** | | | | |
| Pods per cluster | Baluchistan | -0.09 | -0.28** | | | |
| | NA | 0.16** | -0.71** | | | |
| | NWFP | 0.26** | -0.88** | | | |
| | Punjab | 0.50** | 0.21** | | | |
| | Sindh | 0.61** | 0.68** | | | |
| | Un known | 0.61** | 0.87** | | | |
| | Pooled | 0.36** | 0.18* | | | |
| Seed per pod | Baluchistan | 0.08 | -0.08 | 0.41** | | |
| | NA | 0.00 | -0.75** | 0.98** | | |
| | NWFP | 0.15** | -0.71** | 0.93** | | |
| | Punjab | 0.07 | -0.04 | 0.17** | | |
| | Sindh | 0.26** | 0.13* | 0.34** | | |
| | Un known | -0.40** | 0.61** | 0.24** | | |
| | Pooled | -0.35** | -0.37** | 0.20** | | |
| Plant height | Baluchistan | 0.10 | 0.16** | 0.14* | 0.02 | |
| | NA | 0.79** | -0.63** | 0.72** | 0.61** | |
| | NWFP | -0.17** | 0.48** | -0.31** | 0.05 | |
| | Punjab | 0.12* | 0.18** | 0.18** | 0.14* | |
| | Sindh | -0.15** | 0.02 | 0.10 | 0.13* | |
| | Un known | 0.38** | -0.53** | -0.22** | -0.40** | |
| | Pooled | 0.10 | 0.08 | 0.22** | 0.11 | |
| Seed weight | Baluchistan | -0.07 | 0.64 | -0.14* | -0.05 | 0.26** |
| | NA | 0.14* | 0.94 | -0.55** | -0.64** | -0.32** |
| | NWFP | 0.41** | 0.36 | -0.59** | -0.41** | 0.65** |
| | Punjab | -0.40** | -0.07 | -0.54** | -0.21** | -0.20** |
| | Sindh | 0.18** | 0.31 | -0.11 | -0.19** | -0.30** |
| | Un known | -0.33** | 0.53 | 0.20** | 0.97** | -0.41** |
| | Pooled | 0.06 | 0.31** | -0.33** | -0.28** | -0.11 |

*= Significant, **= Highly significant

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