Potential biochemical indicators improve salt tolerance in fifteen cultivars of wheat (*Triticum aestivum L.*) from Pakistan

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Abstract

Proline and glycine-betaine (GB) are the two most important organic osmolytes that hoard in a variety of plant species in response to environmental stresses such as extreme temperatures, drought, salinity, UV radiation and heavy metals. The fifteen cultivars of wheat which are commonly used in Pakistan were subjected to four salt treatments (0 EC, 2 EC, 8 EC, 16 EC) for four weeks and the contents of proline and glycine-betaine were estimated in order to find out the resistant cultivars of wheat against salt stress. Maximum increased production of glycine-betaine was observed in SEHAR-2006 (34.7 µmol /g), LU26-CTR (33.2 µmol/g), NARC-2009 (32.5 µmol/g), BARS-2009 (30.7 µmol/g) and PIRSABAK-09 (30.1 µmol/g) showing obvious tolerance under salt stress at 16 EC. In the control samples the contents of glycine-betaine varied from 5.2 to 6.3 µmol/g. The accumulation of proline in different genotypes showed very close result as of glycine-betaine under salt stress conditions. The calculated amount in SEHAR-06 is (26.98 µmol/g), Lu-26 (26.01 µmol/g), NARC-2009 (25.65 µmol/g), BARC-2009 (25.13 µmol/g) and in PIRSBAK-2009 (25.03 µmol /g). Whereas, the control varieties showed the proline content in the range of 2.54 to 3.75 µmol /g. These results indicate that these cultivars of wheat alleviate the deleterious effect of salt stress by the increased production of proline and betaine.

Keywords: Salt stress, Wheat cultivars, Proline, Glycine-betaine, Biochemical indicators

Introduction

All the environmental factors that effects life processes of the plants are referred to as stresses to plants. There are lots of biotic and a biotic stresses that inhibits productivity and destroys the biomass of the plants. Among these stresses drought, salinity, temperature and more elements are the prominent one that are the barriers in plant production. Immense soil salinity is one of the imperative environmental factors that bound distribution and efficiency of major crops (Ashraf *et al*. 2005; Chandan *et al*. 2006). Agricultural output in arid and semiarid regions of the world is very squat due to accumulation of salts in soils (Ashraf *et al.* 2002; Munns, 2002). Soil salinity causes many bad effects on plant growth, which is due to low osmotic potential of soil solution (osmotic stress), nutritional imbalance, specific ion effects (salt stress), or a combination of these factors (Marschner, 1995; Ashraf, 2004). All these factors cause adverse effects on plant growth and improvement at physiological and biochemical behavior (Ashraf and Sarwar, 2002; Munns and James, 2003).

Wheat is the chief cereal crop of Pakistan, which is cultivated all over the country. It is grown to meet up the food requirement of over growing population of Pakistan. However, per hectare yield of wheat is far beneath than its yield potential, which may be due to different reasons i.e., lack of proper nutrient managements and water, unavailability of fertile soils, drought, salinity, and water logging . In Pakistan, salinity is a serious threat for wheat production. The most of underground water utilized for wheat cropping is brackish; however, some areas are irrigated with canal water but they are short of drainage system both the irrigation systems are increasing the soil salinity problem in the country due to which heavy losses in crop yields are reported (Khan *et al.,* 2006). This is essential to fulfill the wheat grain yield demands of ever growing population of Pakistan. Keeping in view the significance of wheat and salinity, the present study was aimed to estimate the changes that occurred in the biochemical constituents (glycine-betaine and proline) of wheat leaves and to screen out the potential wheat cultivars for better performance under salt stress as the increased levels of proline and glycine-betaine indicates resistant cultivars of wheat.

Materials and methods

The plant material (varieties) was selected on the base of their frequent cultivation in the areas. The selected wheat varieties i.e. LU-26, PASBAN-90, BARS-2009, NARC-2009, FSD-2008, PIRSBAK-90, WAFAQ-2001, LASANI-2008, SEHAR-2006, FAREED-2006, SH-2003, BAHAWALPUR-2000, GA-2002, MERAJ-2008 and SHAFAQ-2006 were collected from different research stations of Pakistan, authenticated and grown in the experimental field of Faculty of Agriculture, Rawalakot Azad Kashmir. The experiment was conducted in plastic pots, filled with equal amount of soil, sand and farm yard manure. The growing media was salinized by commercial NaCl salt to attain salinity level of (control i-e without any dose), 2.0 dS/m (desi Siemen's per meter) 4.0 dS/m, 8.0 dS/m and 16.0 dS/m. The doses of salt were applied at jointing stage and the electrical conductivity (EC) was calculated according to the prescribed method of USDA (1954). Growth observations were recorded at the time of maturity.

1. Proline estimation

The Proline content of the leaves was assessed according to the method of Bates *et al.* (1973). Proline content was calculated from a standard curve, using purified proline as a standard. Results were expressed in μmol/g of fresh weight (FW).

2. Glycinebetaine estimation

The glycine-betaine content was estimated followed by the method of Grieve and Mass (1984). Glycine-betaine content was calculated from a standard curve, using purified glycinebetaine as a standard. Results were expressed in μmol/g of fresh weight (FW).

3. Statistical Analysis

The results were expressed as means ± standard deviation. The data was analyzed by one way ANOVA and different group means were compared by Duncan's multiple range test where necessary. $P < 0.05$ was considered significant in all cases. The software Package statistica was used for analysis of data.

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Results and discussion

Reduced plant growth observed after salt imposition has been reported in glycophytes and even in halophytes (Hamed *et al*. 2007; Panda and Khan, 2009). The negative influence of salinity on the growth might be attributed to salinity inducing effects, osmotic stress, oxidative stress and ionic imbalance (Hasegawa *et al*. 2000), which eventually dent normal cellular metabolism. Choline priming enhanced wheat salt tolerance, in terms of increased shoot and root under NaCl salinity, might be interpreted by the choline possessions on mounting glycinebetaine accumulation, maintaining advantageous elements (K^+, Ca^{2+}), minimizing lethal elements (Na^+, Cl^-) as well as reducing oxidative stress.

Proline and glycine-betaine (GB) are the two most important organic osmolytes that hoard in a variety of plant species in response to environmental stresses such as extreme temperatures, drought, salinity, UV radiation and heavy metals. Even though their definite role in plant osmotolerance is controversial, both compounds are notion to have positive effects on enzyme and membrane integrity that mediates osmotic adjustment in plants which are under stress circumstances. Lot of research work has demonstrated a positive connection between accretion of glycine-betaine and proline and plant stress tolerance, some have argued that the increase in their concentrations under stress is a quantitative product, and not an adaptive response to stress. In present studies, we have discussed the evidence supporting each of these arguments. It is believed under the light of lot of research work that all the plant species are not capable of natural production or accumulation of these compounds when subjected under the stress conditions, extensive research has been conducted investigating various approaches to launch them into plants. Genetically-engineered plants introduced with transgenes for creation of Glycine-betaine or proline have thus far faced with the limitation of being unable to produce adequate amounts of these compounds to improve stress effects.

The estimation of glycine-betaine serves as physiological marker of salt stress. Under saline conditions an overall increasing trend in glycine-betaine contents was noted in all genotypes (Figure 1). Treatment with different doses of salt (2 EC, 4 EC, 8 EC and 16 EC) caused a significant ($P < 0.05$) increase in glycine-betaine. The glycine-betaine contents increased in the order 2 EC < 4 EC < 8 EC < 16 EC. Maximum increased production of glycine-betaine was observed in SEHAR-2006 (34.7 μmol/g), LU-26 CTR (33.2 μmol/g), NARC-2009 (32.5 μmol/g), BARS-2009 (30.7 μmol/g) and PIRSABAK-09 (30.1 μmol/g) showing obvious tolerance under salt stress. In control condition these genotypes were having glycine-betaine contents 6.7 μmol /g, 6.3 μmol /g, 6.1 μmol /g and 6 μmol /g respectively (Figure 1). There was also a significant difference ($P < 0.05$) among different cultivars of wheat for gylcine-betaine. It is obvious in this study that SEHAR-2006 showed excellent results of salt tolerance over all other genotypes and thus it is resistant genotype and sustains well in the soils that are saline in nature up to the maximum salinity level of 16 dS/m. It increases its glycine-betaine production with the increase in salinity level gradually with a linear behavior. Minimum increase was observed in WAFAQ-01 that is 25.7 μmol /g, in MERAJ-2008 that is 26.3 μmol /g and in FAREED-06 that is 27.5 μmol /g. It means there is less production of glycine-betaine in these three genotypes as compared to the rest of genotypes and so these three genotypes are salt sensitive. Thus, the parameter of glycine-betaine production demonstarted these two genotypes not fit for better production in such soil condition which is considered as saline.

Secondly, Proline plays an important role in protecting the sub cellular structures and mediating osmotic adjustment in stressed condition. A positive correlation between the accumulation of these two osmolytes and stress tolerance in plants has been found in many studies. The accumulation of proline in different genotypes showed very close results compared to glycine-betaine under salt stress conditions. The calculated amount in SEHAR-06 is 26.98 µmol /g, in Lu-26 26.01 µmol /g, in NARC-2009 is 25.65 µmol /g, in BARS-2009 is 25.13 µmol /g and in PIRSABAK-2009 is 25.03 µmol /g .This is very good production of proline, as we increased the salinity in all the genotypes and the genotypes in WAFAQ-01 that is 21.11 µmol /g, in MERAJ-2008 that is 23.87 µmol /g and in FAREED-06 that is 23.99 µmol /g showed less production of proline under salt stress conditions (Figure 2). This result also favors the previous result of glycine-betaine production. As we increased the salinity level there is continuous increase in proline production. Glycine-betaine and proline are two important osmolytes that significantly increase under the salt stress. It is concluded that on the basis of osmolytes production, five genotypes viz., SEHAR-2006, LU-26, NARC-2000, BARS-2009 and PIRSBAK-2009 were found to be salt tolerant whereas genotypes,WAFAQ-01, MERAJ-2008 and FAREED-2006 could be designated as sensitive ones. The tolerant genotypes also maintained higher leaf area, osmotic potential, total sugar and chlorophyll contents under saline conditions (data not shown). Several workers have proved that tolerance of plants in their rooting medium is under genetic control (Munns *et al*. 2000). Genetic variabilities are basis for improvement in plants (Akber *et al.* 2009).

Fifteen wheat varieties were grouped into 3 clusters based on proline and glycine-betaine contents under salt stress imposed after seedling stage. Cluster analysis showed that cluster 1 comprised of 4 genotypes, cluster 2 of 6 while cluster 3 contained 5 genotypes. The genotypes in cluster 1 demonstrated higher levels of proline and glycine-betaine contents (Figure 3). One of the check variety LU-26 with known salt tolerance clustered with BARS-09, NARC-09 and SEHAR-06, indicating a group of salt tolerant varieties. Similarly, the 2nd cluster comprised of another salt tolerant check variety PASBAN-90. The level of proline and glycine-betaine contents among the members of this cluster was slightly lower than the members of first cluster indicating moderate level of tolerance to the salt stress. Cluster 3 comprised of the varieties showing lowest level of proline and glycine-betaine contents hence lowest tolerance to the salt stress. The dendrogram also depicted the sequential ability of the varieties to show tolerance to salt stress, as the level of tolerance decreased from top to bottom in the figure. Hence LU-26 and BARS-09 were the most tolerant varieties while SHAFAQ-06 and SH-03 were most susceptible varieties among the material studied.

In conclusions, the examined wheat varieties exhibited a significant difference in their glycine-betaine and proline contents showing the biochemical variation among different genotypes. Certain wheat varieties, SEHAR-2006, LU-26, NARC-2009, BARS-2009 and PIRSABAK were found to contain high concentrations of proline and glycine-betaine. In addition some other wheat varieties, SHAFAQ-06, LASANI-08 and GA-2002 also exhibit high amount of biochemical indicators. However, further detailed studies are required to evaluate the effect of salt stress on antioxidant enzymes and molecular studies.

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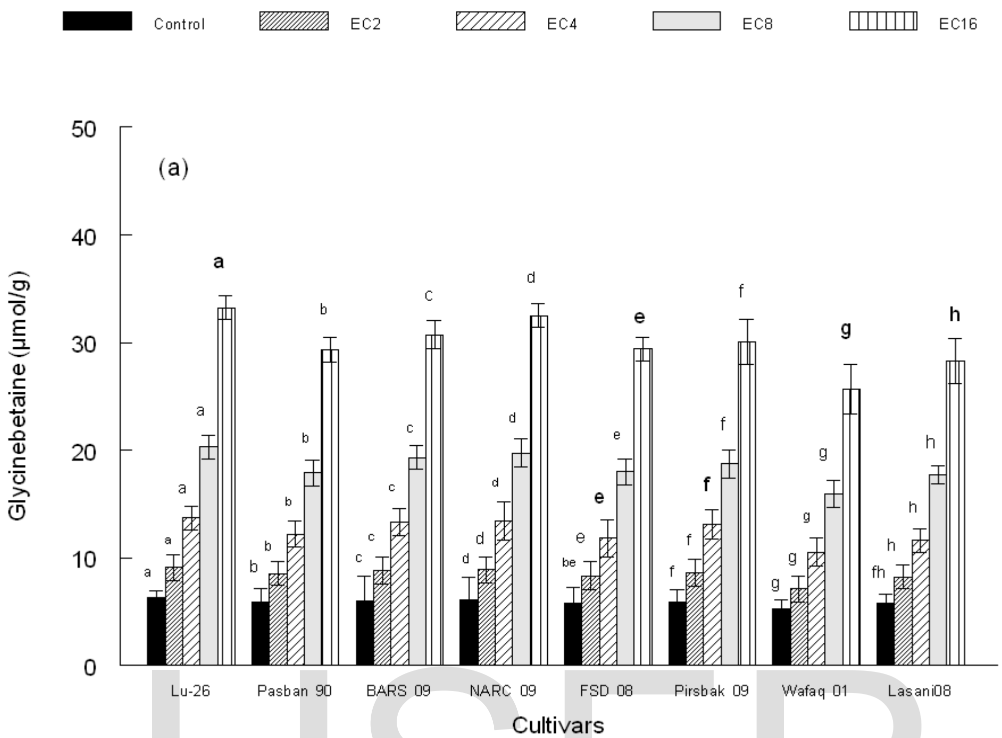
Figure Legends

Fig. 1. The effect of salt stress on glycine-betaine content in different cultivars of wheat.

Values are mean ± SD (n=3). The salt stress causes a significant increase ($p < 0.05$) in glycine betaine at different doses (Control, 2 EC, 4 EC, 8 EC and 16 EC) of salt. Mean values of glycine-betaine among different genotypes of wheat with different letters are significantly different ($P < 0.05$) from each other by DMR test.

Fig. 2. The effect of salt stress on proline content in different cultivars of wheat. Values are mean ± SD (n=3). The salt stress causes a significant increase ($P < 0.05$) in proline at different doses (Control, 2 EC, 4 EC, 8 EC and 16 EC) of salt. Mean values of proline among different genotypes of wheat different letters are significantly different ($P < 0.05$) from each other by DMR test.

Fig. 3. Dendogram of cluster analysis for proline and glycinebetaine contents in different cultivars of wheat.


Control
EC2
EC4
EC8
EC16
(a)
Glycinebetaine (µmol/g)
Cultivars
Lu-26
Pasban 90
BARS 09
NARC 09
FSD 08
Pirsbak 09
Wafaq 01
Lasani08

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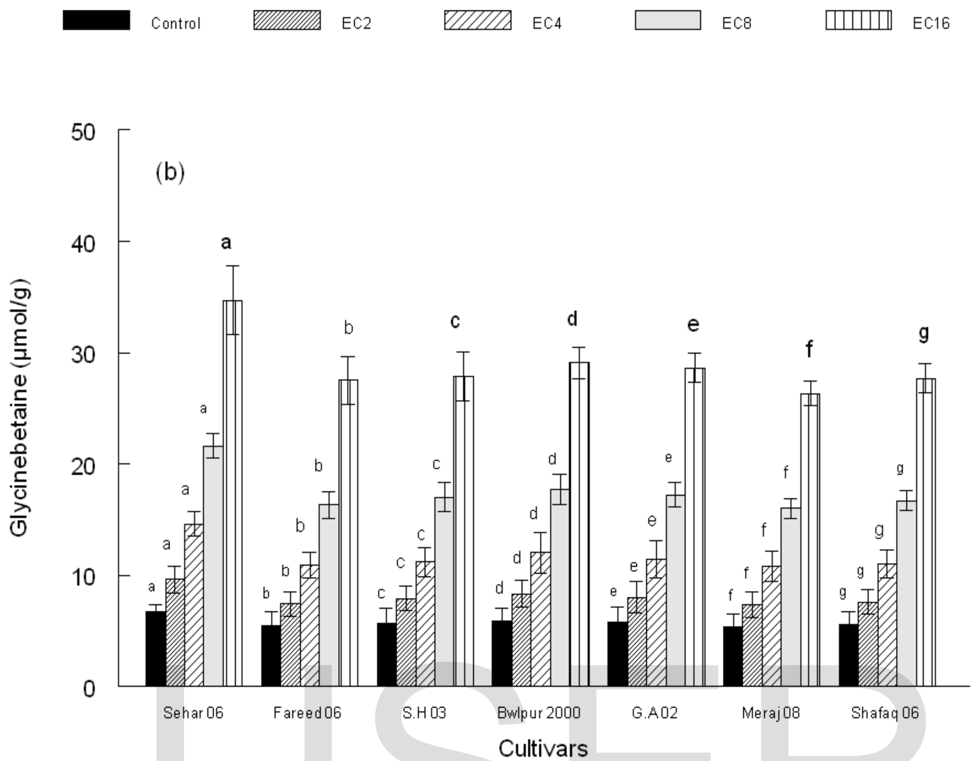
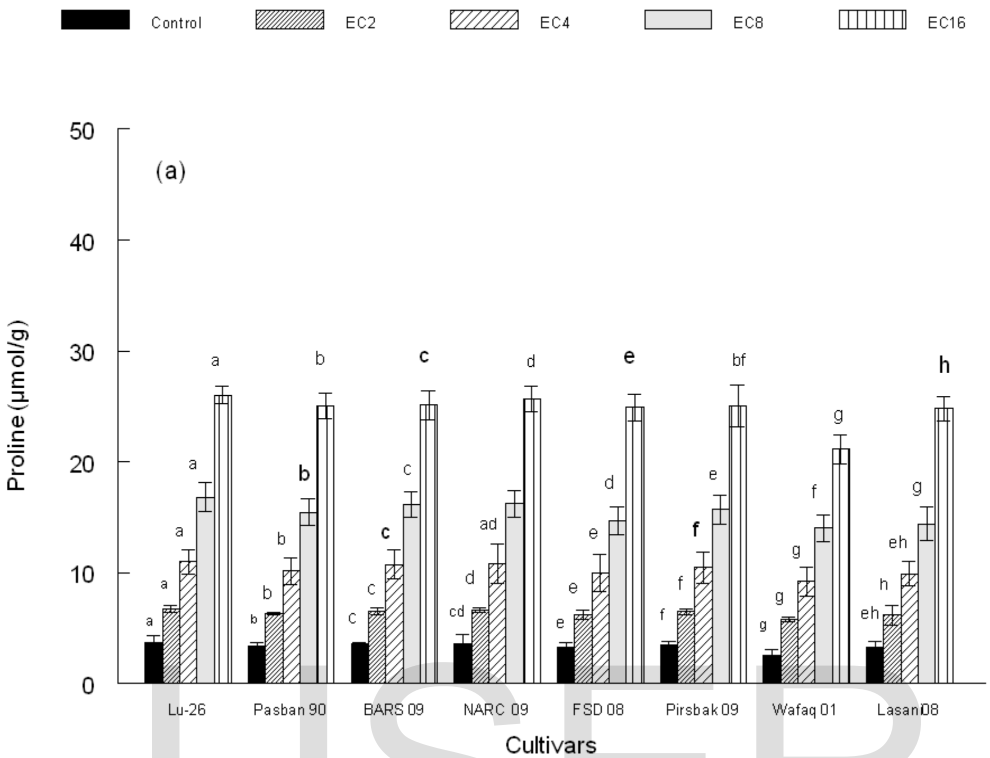


Fig. 1


Control
EC2
EC4
EC8
EC16
(a)
Proline (µmol/g)
50
40
30
20
10
0
Lu-26
Pasban 90
BARS 09
NARC 09
FSD 08
Pirsbak 09
Wafaq 01
Lasani 08
Cultivars

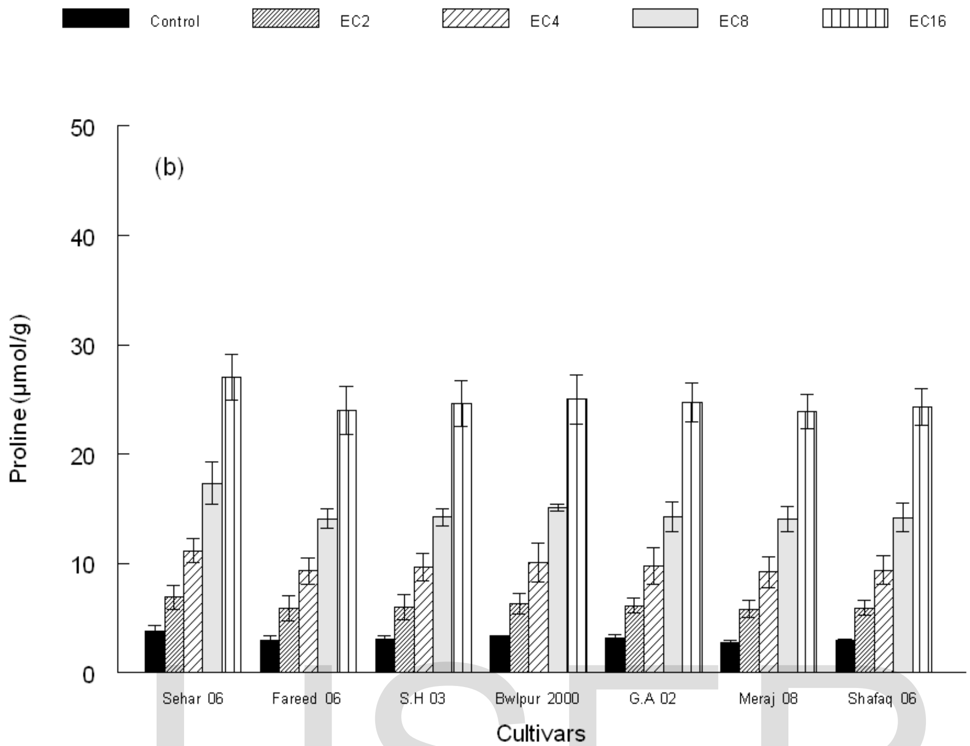

Control
EC2
EC4
EC8
EC16
(b)
Proline (µmol/g)
0
10
20
30
40
50
Sehar 06
Fareed 06
S.H 03
Bwlpur 2000
G.A 02
Meraj 08
Shafaq 06
Cultivars

Fig. 2

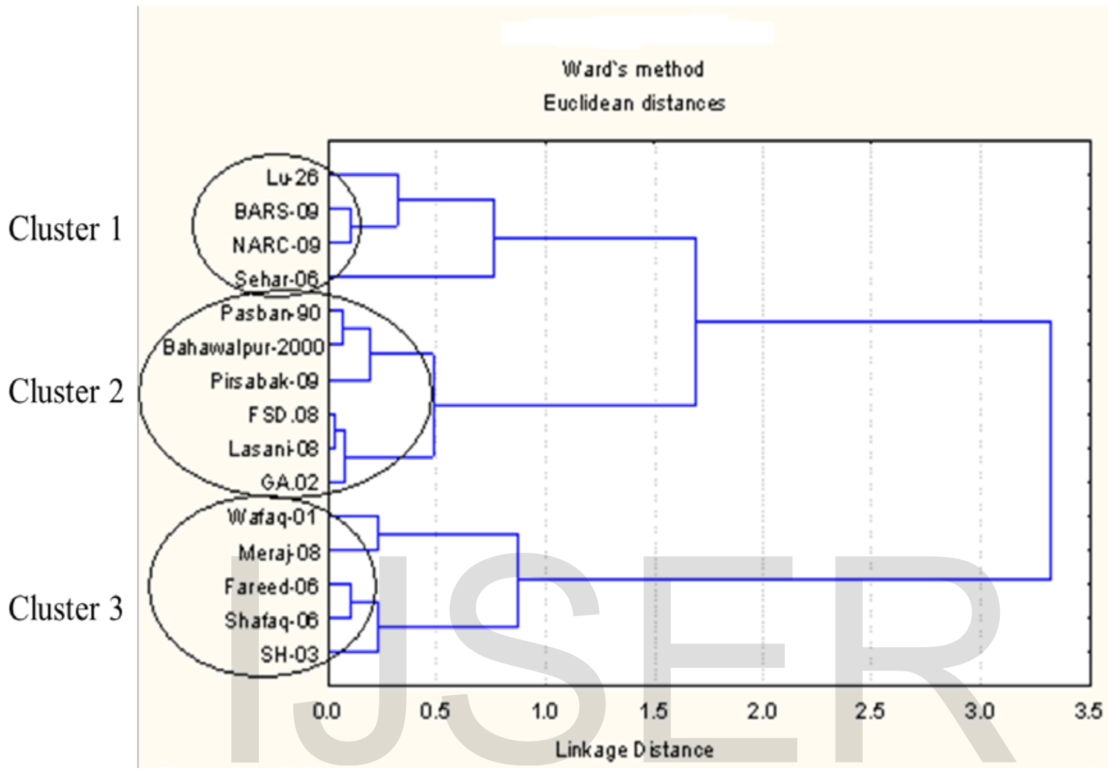

Ward`s method
Euclidean distances
Cluster 1
Cluster 2
Cluster 3
Lu-26
BARS-09
NARC-09
Sehar-06
Pasban-90
Bahawalpur-2000
Pirsabak-09
FSD.08
Lasani-08
GA.02
Wafaq-01
Meraj-08
Fareed-06
Shafaq-06
SH-03
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Linkage Distance

Fig. 3.