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# NONPARAMETRIC STABILITY ANALYSIS IN MULTI-ENVIRONMENT TRIAL OF CANOLA

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## ABSTRACT

**Rapeseed is the world's second most important source of vegetable oils. Development of genotypes having high seed yield with stable performance is of paramount importance. In the present investigation seventeen genotypes were grown at seven locations during two growing seasons in semi-cold regions of Iran. Data recorded on seed yield were subjected to different nonparametric measures which do not require distributional assumptions. Results of nonparametric tests of G, E and GE interaction and a combined ANOVA across environments showed there were both cross over and non-cross over interactions for G and E and only non-cross over type for GE interaction. In this study, high values of Top (proportion of environments in which a genotype ranked in the top third) and mean of rank were associated with high mean yield. However Rank-sum measure was successful to detect genotypes showing simultaneous high yield and yield stability. Cluster analysis and principal component (PC) analysis help to group genotypes and measures and they revealed association among different statistics. Finally, among nonparametric measures, Top, $S_i^{(1)}$ and Rank-sum statistics of nonparametric procedures were found to be useful in detecting the stability of the genotypes. According to these parameters Geronimo was found as stable and high yield canola genotype.**

**Keywords:** ***Brassica napus*, Multi-environment trial, nonparametric measures.**

## INTRODUCTION

Seeds from rapeseed (*Brassica napus* L.) are used for oil extraction, which makes rapeseed the world's second most important source of vegetable oils after soybean (FAO,2012).During these years, the importance of rapeseed has significantly increased in Iran, mainly due to the demand of oil and some transport sanctions. Worldwide canola production is approximately 50 million tons and covers a total area of 27 million hectares (FAOSTAT, 2010). The total area of canola in Iran in the 2010-2011 season was 93000 ha which produced 190000 tons rapeseed (Agricultural Ministry Statistics, 2011). Crops are largely influenced by climate conditions during growing season; hence, even minor climate changes may cause highly variation on crop yield. The environment is the all surrounding conditions including location and year. Any difference in location properties such as soil texture, pH, depth, organic matter, and so on affect cultivar growth and development. The genotype × environment interaction (GEI) is the response of each cultivar to variations in the environment Crossa et al. (1991). The GE interaction has been one of the principal subjects of study in breeding, allowing the generation of different methodologies for genetic improvement. It is perquisite to evaluate a cultivar behavior during different years and locations to find out cultivars with general or specific adaptation before release or any recommendation. There are two major parametric and non-parametric approaches to study GE interaction and determining adaptation of genotypes (Huehn, 1996). A parametric approach relies on large number of assumptions about genotypic, environmental, and GE interaction like normal distribution of errors and interaction effects. Any change in these assumptions may give inaccurate results (Huehn, 1990a). Nonparametric Stability Analysis (NPSA) based on ranks are far from any specific assumptions and provide an alternative useful approach (Huehn, 1990a). These methods provide a lot of flexibility for plant breeders for the simultaneous selection for both mean yield and stability (Karimizadeh et al., 2012). The nonparametric statistics are easy to use and appears to be able to extract a large portion of the GE interaction. Meanwhile, interpretation of GE interaction and yield stability is feasible in different crops using nonparametric strategy. Several nonparametric measures and later modifications proposed till now (Hühn, 1979; Hühn and Nassar, 1989; Kang and Pham, 1991; Lu, 1995; Nassar and Huehn, 1987; Thennarasu, 1995). These parameters are mainly based on the ranks, $r_{ij}$, or adjusted ranks, $r^*_{ij}$, of genotypes in each environment. The major criterion to classify a genotype as stable is similar rankings across environments. Some measures consider only stability of genotypes like $S_i^{(3)}$ and $S_i^{(4)}$ while others like $S_i^{(1)}$, $S_i^{(2)}$ and Kang's (1988) rank-sum combine both yield and stability to propose an ideal genotype (Kang and Pham, 1991). In practice, the ideal cultivar would be the one with high seed yield and high stability when evaluated across different environments (Yan et al., 2007). Thennarasu (1995) proposed the four non-parametric stability measures, $NP_i^{(1)}$, $NP_i^{(2)}$, $NP_i^{(3)}$ and $NP_i^{(4)}$ based on adjusted rank calculated from adjusted mean yield ($x^*_{ij} = x_{ij} - \bar{x}_{i.}$). In this case, the rank removes the effect of the environment (E) and genotypes (G) and is only depend on GE and error effects. There are two different types of genotype × environment interaction, conceptually (Baker, 1988). Cross over (or quantitative) type of interaction referred to change in rank orders of genotypes across environments while non-cross over (or qualitative) interaction referred to difference in rank amounts of genotypes. The breeders are mainly interested in the former type. Some procedures have been developed to reveal the type of GE interaction (Azzalini and Cox, 1984; Bredenkamp, 1974; Hildebrand, 1980; Kroon and Laan, 1981; Kubinger, 1986). In the methods of Bredenkamp (1974), Hildebrand (1980) and Kubinger (1986), interactions are defined as deviations from the additivity of main effects for genotypes and environments. In the other hand, using these methods non-cross over interactions are defined. The methods of Kroon and Laan (1981) and Azzalini and Cox (1984) define interactions according to the cross over interactions model. So, using these methods cross over GEI is evaluated. Since nonparametric test is robust to ANOVA assumptions results obtained from these methods can be more reliable (Truberg and Hühn, 2002). Association among stability measures helps breeder to choose the best and most informative method obtaining reliable prediction of cultivar behaviors and also to fit the statistic and dynamic concepts of stability (Sabaghnia et al., 2006).

The objectives of this study were to (i) identify canola genotypes that have both high yield and stability across different test environments of Iran's cold and semi cold areas, (ii) apply nonparametric tests to investigate the cross over and non-cross over GE interaction in multi-environment trials, and (iii) study the relationships among different nonparametric stability statistics.

## MATERIALS and METHODS

### *Plant material*

17 winter rapeseed cultivars were used as plant material. Cultivars were grown in 14 environments (seven locations during two growing seasons). The experimental layout was randomized complete block design with four replications in each environment. The seed was sown in four rows, 5 m long, with a between-row spacing of 30 cm. All agricultural practices were applied based on recommended protocols for all locations. Grain yield of each cultivar was recorded on a plot basis.

### *Nonparametric Stability Analysis (NPSA)*

A parametric combined analysis of variance (ANOVA) (F-test) and three non-parametric statistical procedures including Kroon and Laan (1981), Bredenkamp (1974) and Hildebrand (1980) were used to test the significance of GEI, G and E (Hühn and Léon, 1995). The test statistics for rank-change interactions, genotypes and environments are approximately $x^2$ distributed with ($l$-1) × ($m$-1), ($l$-1) and ($m$-1) degrees of freedom, respectively, where $l$ is the number of genotypes, and $m$ the number of environments (Hühn and Léon, 1995). For a two-way dataset with $k$ genotypes and $n$ environments, it was denoted the phenotypic value of $i$th genotype in $j$th environment as $x_{ij}$, where $i=1,2,\ldots,k;$ $j=1,2,\ldots,n;$ $r_{ij}$ as the rank of the $i$th genotype in the $j$th environment, and $\bar{r}_{ij}$ as the mean rank across all environments for the $i$th genotype. Huehn (1990b) proposed a corrected mean yield on the nonparametric measures consists on $S_i^{(1)}$ and $S_i^{(2)}$ to remove the effect of genotype from phenotypic value. $S_i^{(3)}$ and $S_i^{(6)}$ are the sum of the absolute deviations and sum of squares of ranks, respectively. The genotype with the highest adjusted yield was given a rank of 17 and a genotype with the lowest adjusted yield was assigned a rank of 1. A genotype is stable over environments if its ranks are similar over environments; i.e. maximum stability occurs with equal ranks over environments.

The formula for calculating the statistics based on yield ranks of genotypes in each environment are expressed as follows (Hühn, 1979):

$$S_i^{(1)} = \sum_{j=1}^{n-1}\sum_{j'=j+1}^{n} |r_{ij} - r_{ij'}| / [n(n-1)]$$

$$S_i^{(2)} = \frac{\sum_{j=1}^{n} (r_{ij} - \bar{r}_{i.})^2}{n-1}$$

$$S_i^{(3)} = \frac{\sum_{j=1}^{n} (r_{ij}^{*} - \bar{r}_{i.}^{*})^2}{\bar{r}_{i.}^{*}}$$

$$S_i^{(4)} = \sqrt{\frac{\sum_{j=1}^{n} (r_{ij} - \bar{r}_{i.})^2}{n}}$$

$$S_i^{(5)} = \frac{\sum_{j=1}^{n} |r_{ij} - \bar{r}_{i.}|}{n}$$

Where $r_{ij}^*$ are ranks of genotypes in each environment based on corrected mean and $r_{ij}$ are ranks of genotypes in each environment based on the mean yields, $X_{ij}$.

Fox et al. (1990) suggested nonparametric superiority index (SI), Top, Mid and Low. They expressed widely adapted genotypes fall into top third section of ranks after stratified ranking of the genotypes at each environment. A genotype with high value Top is considered as a widely adapted genotype. Top, Mid and Low are the proportion of ranks the genotype occurs in the top third, middle third and bottom third of the ranks.

$$S_i^{(6)} = \frac{\sum_{j=1}^{n} |r_{ij}^* - \bar{r}_{i.}^*|}{\bar{r}_{i.}^*}$$

Kang's (1988) rank-sum is another nonparametric stability statistics where both the genotype mean rank and Shukla's (1972) stability variance are used as selection criteria. This statistics assigns a weight of one to both mean yield and stability and enables the identification of high yielding and stable genotype, simultaneously.

Thennarasu (1995) proposed the use of four nonparametric measures based on the corrected ranks of genotypes in each environment. The ranks of genotypes in each environment were adjusted as ($x_{ij}^* = x_{ij} - \bar{x}_{i.}$).

Thennarasu's (1995) nonparametric stability statistics are:

$$NP_i^{(1)} = \frac{\sum_{j=1}^{n} |r_{ij}^* - Md_i^*|}{n}$$

$$NP_i^{(2)} = \frac{\sum_{j=1}^{n} |r_{ij}^* - Md_i^*| / Md_i}{n}$$

$$NP_i^{(3)} = \frac{\sqrt{\sum_{j=1}^{n} (r_{ij}^* - \bar{r}_{i.}^*)^2 / n}}{\bar{r}_{i.}^*}$$

$$NP_i^{(4)} = \frac{2}{n(n-1)} \left[ \sum_{j=1}^{n-1} \sum_{j'=j+1}^{n} |r_{ij}^* - r_{ij'}^*| / \bar{r}_{i.} \right]$$

In the above formulas, $r_{ij}^*$ is the rank of $X^*$ and $M_{di}^*$ are the mean and median ranks for adjusted values. Spearman's coefficient of rank correlation was employed (Steel and Torrie, 1980) to statistically compare the stability indices used in this study. To understand relationships among stability methods, principal component analysis (PCA) based on rank correlation matrices and to group different parameters into clusters, hierarchical cluster analysis with squared Euclidean distance and Ward's method were performed using IBM SPSS Statistics 21. All nonparametric stability statistics calculated using SAS-based program, and Microsoft Excel (Hussein et al., 2000; Lu, 1995).

## RESULTS AND DISCUSSION

### *Analysis of variance and nature of GE interaction*

The parametric combined analysis of variance indicated that the main effects of genotypes, environments, locations and years were highly significant (Table 1). Also, all the second order interactions including the GE interaction effect was significant ($P < 0.01$). The significant genotype × location interaction implied that the responses of the genotypes changed depending on the environmental conditions. These finding are in agreement with the results reported by Balalić and Zorić (2013). There was no significant genotype × year interaction that means genotype behavior in a location between years is not significant. The large seed yield variation due to environment is the main source of variation in most of the multi-environment trials (Gauch and Zobel, 1997). In present study, the canola seed yield was affected by environment, which accounted for 91 % of sum of squares (E+G+GE), whereas G and GE captured 2% and 7% of sum of squares (E+G+GE), respectively. Normally, environment explains most (80% or higher) of the total yield variation, while genotype and GE interaction are usually small (Yan and Kang, 2003). The results in this investigation were in agreement with other reports of multi-environment yield trials. However, effective interpretation and utilization of a multi-environment trial dataset in making selection decisions remains a major challenge to researchers.

**Table 1.** Combined analysis of variance of 17 canola genotypes across 14 environments

| Source of variation | d.f. | Sum of squares | Mean of squares | (%) Explained |
|---|---|---|---|---|
| Environments (E) | 13 | 1371.82 | 105.52 $^{**}$ | 90.84 |
| Place (P) | 7 | 1126.76 | 160.96 $^{**}$ | |
| Year (Y) | 1 | 14.57 | 14.57 $^{**}$ | |
| P×Y | 5 | 242.95 | 48.59 $^{**}$ | |
| Rep (P×Y) | 41 | 34.75 | 0.84 $^{**}$ | |
| Genotypes (G) | 16 | 30.88 | 1.93 $^{**}$ | 2.04 |
| G×E | 208 | 107.34 | 0.51 $^{**}$ | 7.10 |
| G×P | 112 | 57.80 | 0.51 $^{**}$ | |
| G×Y | 16 | 6.52 | 0.40 $^{ns}$ | |
| G×P×Y | 80 | 42.37 | 0.52 $^{*}$ | |
| Error | 656 | 246.79 | 0.37 | |
| Total | 934 | 1796.63 | | |

$^{**}$ Significant at the 0.01 probability level; $^{ns}$ Non-Significant at the 0.05 probability level

The test statistics of the different nonparametric statistical procedures including Bredenkamp (1974) and Hildebrand (1980) for non-cross over GE interaction indicated the presence of this interaction type in canola dataset. Kroon and Laan (1981) procedure showed that there was only non-cross over or non-additive GE interaction type in this investigation (Table 2). Lack of cross over interaction for quantitative traits even in the presence of significant GE interaction has been reported (Kang, 2002). Although these findings are in agreement with the conventional ANOVA, but provide more specific information about the nature of GE interactions. This advantage is also noted in studies of Karimzadeh et al. (2013) and Sabaghnia et al., (2013). Cooper and Byth (1996) described that the larger the degree of GEI, the more dissimilar the genetic systems controlling the physiological processes conferring adaptation to different environments. The complexity of genotype behavior in different environments make difficulties to have selection in any specific environment (Karimzadeh et al., 2013; Sabaghnia et al., 2008; Yau, 1995). The value ratio obtained by the application of non-parametric methods is not in agreement with the ratio presented by Huehn (1996), considering that the $x^2$-values of Bredenkamp had the lowest numerical values in all tested traits. Delic et al., (2009) was also found disagreement in Kroon and Laan method with Huhn results. Non-cross over interactions may mean that genotypes are genetically heterogeneous but test environments are more or less homogeneous or that genotypes are genetically homogeneous but environments are heterogeneous (Kang, 2002).

**Table 2.** Test statistics for a test of genotype × environment interaction of 17 Canola genotypes over 14 environments.

| Nonparametric test | d.f. | Statistic $\chi^2$ |
|---|---|---|
| van der Laan-de Kroon | 208 | 214.33 $^{ns}$ |
| Bredenkamp | 208 | 5626.86 $^{**}$ |
| Hildebrand | 208 | 255.44 $^{*}$ |

$^{*}$, $^{**}$ Significant at the 0.05 and 0.01 probability levels, respectively; $^{ns}$ Non-Significant at the 0.05 probability level

The relatively large magnitude of GE interaction for grain yield of 17 canola genotypes tested across seven locations were larger than that of genotypic main effect (three times), but smaller than that of environment main effect (Table 1). The studied genotypes showed cross over types of GE interaction while environments showed both cross over and non-cross over types of GE interaction (Table 3). The relative contributions of G and GE interaction effects to the total variation for grain yield found in this study are in agreement with those found in other crop adaptation studies such as cereals or food legumes in rain-fed environments (Alagarswamy and Chandra, 1998; Balalić et al., 2011; Ceccarelli and Grando, 1991; Mohammadi et al., 2007; Sabaghnia et al., 2008; Živanović et al., 2012). Therefore, it would be very difficult to achieve an indirect response to selection over all of the canola target population of environments from selection in a few environments, ignoring the observed GE interactions.

**Table 3.** Test statistics for a test of genotype and environments of 17 Canola genotypes over 14 environments

| Nonparametric test (G) | d.f. | Statistic $\chi^2$ |
|---|---|---|
| van der Laan-de Kroon | 16 | 35.46 $^{**}$ |
| Bredenkamp | 16 | 20.63 $^{ns}$ |
| Hildebrand | 16 | 0.56 $^{ns}$ |
| Nonparametric test (E) | d.f. | Statistic $\chi^2$ |
| van der Laan-de Kroon | 13 | 1289.48 $^{**}$ |
| Bredenkamp | 13 | 644.49 $^{**}$ |
| Hildebrand | 13 | 2954.04 $^{**}$ |

$^{**}$ Significant at the 0.01 probability level; $^{ns}$ Non-Significant at the 0.05 probability level

### *Nonparametric Stability Analysis (NPSA)*

According to mean yield, genotype Ebonite (3410 kg ha$^{-1}$), Elite (3190 kg ha$^{-1}$) and ARC-5 (3316 kg ha$^{-1}$) were the high yielding genotypes (Table 4). It has been suggested that, in unfavorable environments, plant breeders should look at GE interaction in a different way (Stroup et al., 1993). Yield stability is more important than mean yield in these environments. According to Ceccarelli (1996), in an unfavorable environment, the lowest mean yield of landraces was always higher than the lowest mean yield of non-landraces. This particular property of landraces is identifiable as stability by farmers but it would have been missed in a selection process within a breeding program to select landraces for unfavorable environments (Ceccarelli, 2000). According to the mean of ranks, ARC-5 and Ebonite showed highest yield over environments while regarding to corrected mean of ranks, Licord and Opera were the top rank. The effect of correction and removing the genotype effect from phenotypic data is in agreement with Karimizadeh et al. (2012). Ketata *et al.* (1989) proposed two ranking methods according to mean and standard deviation of ranks and Cravero *et al.* (2010) reported advantages of these nonparametric procedures in phenotypic stability studies. According to corrected and uncorrected CV nonparametric measures contain mean and standard deviation of ranks, genotype Geronimo identified as the high stable genotype (Table 3). Genotypes Geronimo and Sunday with minimum first two nonparametric stability statistics of Huehn (1990a) which are known as $S_i^{(1)}$ and $S_i^{(2)}$ were the most stable genotypes (Table 4). The $S_i^{(1)}$ estimates are based on all possible pair-wise rank differences, while $S_i^{(2)}$ is based on variances of ranks for each genotype across environments (Hühn and Nassar, 1989; Nassar and Huehn, 1987). These statistics ranked genotypes similarly for stability. The similarity result of these two parameters was also found in Balalić and Zorić (2013). Genotypes Licord, Okapi, Elite and Ebonite were most unstable. The significance tests for $S_i^{(1)}$ and $S_i^{(2)}$, developed by Nassar and Huehn (1987). For each canola genotype, $Z_i^{(1)}$ and $Z_i^{(2)}$ values were calculated according to the ranks of adjusted data and summed over genotypes to obtain *Z* values. Since $Z_i^{(1)}$ sum = 30.72 and $Z_i^{(2)}$ sum = 35.81 were more than the critical value of $x^2$ sum = 27.58, there were significant differences among 17 canola genotypes across 14 environments (Table 2). Inspecting the individual Z values, it was found that some genotypes were significantly unstable relative to others, because they revealed large Z values, in comparison with the critical value $X^2_{0.05,df=1}$ = 3.84. For practical applications Huehn (1990b), preferred the use of $S_i^{(1)}$ against $S_i^{(2)}$.

**Table 4 .** Estimation and test of nonparametric stability measures for 17 Canola genotypes across environments

| **Genotype** | **Mean Yield (Kg ha$^{-1}$)** | **MR** | **CMR** | **SD** | **CSD** | **CV** | **CCV** | $S_i^{(1)}$ | $Z_i^{(1)}$ | $S_i^{(2)}$ | $Z_i^{(2)}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ebonite | 3410.00 | 13.64 | 8.14 | 3.89 | 6.00 | 0.29 | 0.74 | 7.033 | 3.227 | 35.978 | 3.688 |
| Elite | 3190.50 | 10.93 | 8.00 | 5.17 | 6.05 | 0.47 | 0.76 | 6.956 | 2.879 | 37.456 | 4.654 |
| Sinatra | 2908.07 | 7.79 | 9.36 | 3.70 | 4.13 | 0.48 | 0.44 | 4.868 | 1.019 | 17.017 | 1.254 |
| Sahara | 2950.36 | 7.00 | 8.64 | 4.64 | 4.92 | 0.66 | 0.57 | 5.725 | 0.010 | 24.247 | 0.002 |
| Celsius | 2941.00 | 7.14 | 8.86 | 4.75 | 4.42 | 0.67 | 0.50 | 5.143 | 0.427 | 19.517 | 0.517 |
| Sunday | 3003.14 | 8.79 | 8.93 | 3.87 | 3.89 | 0.44 | 0.44 | 4.637 | 1.713 | 15.824 | 1.718 |
| Modena | 3040.86 | 9.43 | 9.29 | 4.72 | 4.75 | 0.50 | 0.51 | 5.604 | 0.003 | 22.528 | 0.056 |
| Geronimo | 3059.64 | 10.07 | 8.43 | 3.10 | 3.32 | 0.31 | 0.39 | 3.692 | 6.419 | 11.033 | 4.322 |
| Opera | 2666.64 | 4.86 | 10.36 | 3.30 | 5.75 | 0.68 | 0.55 | 6.670 | 1.759 | 33.017 | 2.090 |
| ARC-5 | 3316.43 | 12.57 | 8.07 | 5.00 | 6.01 | 0.40 | 0.74 | 6.967 | 2.927 | 35.209 | 3.230 |
| ARC-2 | 2969.21 | 8.29 | 8.79 | 4.53 | 4.35 | 0.55 | 0.50 | 5.044 | 0.611 | 18.951 | 0.655 |
| Milena | 3092.50 | 11.07 | 9.50 | 4.57 | 4.59 | 0.41 | 0.48 | 5.374 | 0.126 | 21.039 | 0.225 |
| Dexter | 2870.93 | 7.21 | 9.86 | 4.61 | 4.74 | 0.64 | 0.48 | 5.538 | 0.020 | 22.440 | 0.063 |
| SLM046 | 3036.71 | 9.79 | 8.64 | 3.70 | 4.29 | 0.38 | 0.50 | 4.967 | 0.777 | 17.758 | 1.002 |
| Zarfam | 3111.29 | 11.14 | 8.29 | 4.00 | 4.46 | 0.36 | 0.54 | 5.297 | 0.206 | 19.912 | 0.430 |
| Okapi | 2810.36 | 6.29 | 9.64 | 5.88 | 6.07 | 0.93 | 0.63 | 7.110 | 3.595 | 36.863 | 4.253 |
| Licord | 2772.57 | 6.86 | 10.21 | 5.72 | 6.42 | 0.83 | 0.63 | 7.374 | 5.008 | 41.258 | 7.656 |
| | | | | | | | | | | Test Statistics | |
| | Grand Mean | | | $E(S_i^{(1)})$ | $E(S_i^{(2)})$ | $Var(S_i^{(1)})$ | $Var(S_i^{(2)})$ | $x^2$ $Z_1$,$Z_2$ | | $x^2$ sum | |
| | 3008.84 | | | 5.647 | 24 | 0.595 | 38.901 | 8.844 | | 27.587 | |

**Table 4.** Continue

| Genotype | $S_i^{(3)}$ | $S_i^{(6)}$ | $NP_i^{(1)}$ | $NP_i^{(2)}$ | $NP_i^{(3)}$ | $NP_i^{(4)}$ | PI | ProbP | TOP | MID | LOW | RS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ebonite | 55.00 | 10.13 | 5.29 | 0.34 | 1.226 | 0.135 | 0.02 | 0.96 | 85.71 | 14.29 | 0.00 | 9 |
| Elite | 49.06 | 8.93 | 5.29 | 0.42 | 1.237 | 0.172 | 0.08 | 0.43 | 42.86 | 42.86 | 14.29 | 17 |
| Sinatra | 17.46 | 4.17 | 3.50 | 0.58 | 1.086 | 0.086 | 0.27 | 0.00 | 14.29 | 14.29 | 71.43 | 20 |
| Sahara | 25.45 | 4.73 | 3.79 | 0.54 | 1.141 | 0.102 | 0.27 | 0.00 | 14.29 | 14.29 | 71.43 | 26 |
| Celsius | 26.55 | 5.10 | 3.43 | 0.53 | 1.110 | 0.093 | 0.25 | 0.00 | 28.57 | 42.86 | 28.57 | 17 |
| Sunday | 20.31 | 4.66 | 2.93 | 0.31 | 1.085 | 0.064 | 0.17 | 0.04 | 0.00 | 71.43 | 28.57 | 12 |
| Modena | 33.77 | 6.43 | 3.71 | 0.37 | 1.115 | 0.090 | 0.19 | 0.02 | 42.86 | 28.57 | 28.57 | 16 |
| Geronimo | 15.76 | 4.41 | 2.43 | 0.24 | 1.070 | 0.057 | 0.15 | 0.07 | 28.57 | 57.14 | 14.29 | 8 |
| Opera | 11.54 | 2.77 | 4.93 | 1.10 | 1.134 | 0.106 | 0.48 | 0.00 | 0.00 | 14.29 | 85.71 | 30 |
| ARC-5 | 59.95 | 10.63 | 5.21 | 0.35 | 1.231 | 0.143 | 0.07 | 0.52 | 85.71 | 0.00 | 14.29 | 18 |
| ARC-2 | 27.47 | 5.50 | 3.36 | 0.35 | 1.108 | 0.091 | 0.22 | 0.01 | 14.29 | 42.86 | 42.86 | 11 |
| Milena | 39.10 | 7.03 | 3.79 | 0.32 | 1.103 | 0.096 | 0.16 | 0.05 | 42.86 | 42.86 | 14.29 | 15 |
| Dexter | 25.62 | 5.10 | 3.86 | 0.55 | 1.102 | 0.089 | 0.34 | 0.00 | 14.29 | 42.86 | 42.86 | 25 |
| SLM046 | 21.50 | 5.44 | 3.64 | 0.40 | 1.108 | 0.097 | 0.17 | 0.04 | 28.57 | 42.86 | 28.57 | 12 |
| Zarfam | 30.29 | 6.08 | 3.71 | 0.31 | 1.127 | 0.106 | 0.13 | 0.11 | 28.57 | 71.43 | 0.00 | 10 |
| Okapi | 38.52 | 5.98 | 5.21 | 1.30 | 1.170 | 0.163 | 0.46 | 0.00 | 28.57 | 0.00 | 71.43 | 32 |
| Licord | 38.21 | 6.10 | 5.50 | 0.85 | 1.169 | 0.096 | 0.41 | 0.00 | 0.00 | 57.14 | 42.86 | 28 |

MR = Mean of Rank; CMR = Corrected Mean of Rank; SD=Standard Deviation; CSD= Corrected Standard Deviation; CV= Coefficient of Variation; CCV= Corrected Coefficient of Variation; PI = Superiority measure of Lin and Binns (1988); ProbPI= The significance level of the test on PI; TOP, MID, and LOW are the parameters of Fox et al. (1990); RS = the Rank-Sum of Kang (1988)

This stability parameter is very easy to compute and allows a clear and relevant interpretation (mean absolute difference between environments). Furthermore, an efficient test of significance is also available. Non-parametric statistics of $S_i^{(3)}$ and $S_i^{(6)}$ combine yield and stability based on yield ranks of genotypes in each environment (Hühn, 1979). Genotypes Opera, Geronimo and Sinatra based on $Si^{(3)}$ and $Si^{(6)}$ statistics were identified as the most stable genotypes (Table 4). The numerical value of $Si^{(3)}$ is determined by both yield and stability. Among these the most stable genotypes, genotype Geronimo had relatively high mean yield (Table 4). According to Nassar and Huehn (1987), Flores et al. (1998), Scapim et al. (2000) and Segherloo Ebadi et al. (2008), $S_i^{(1)}$ and $S_i^{(2)}$ nonparametric stability statistics considered in the sense of homeostasis and show static or biological concept of stability. Sabaghnia et al. (2006), reported that $S_i^{(3)}$ and $S_i^{(6)}$ nonparametric stability statistics are associated with the static or biological concept of stability. Based on Top measure, genotypes Ebonite and ARC-5 were the most favorable genotypes while according to Mid measure, genotypes Sunday and Zarfam were detected the most favorable genotypes (Table 4). Considering all three Top, Mid and Low values as the nonparametric superiority index (PI) of Fox et al. (1990), genotypes Ebonite, Elite and ARC-5 were the most favorable genotypes from both stability and mean yield aspects. Significance level (p-value) for superiority measure of each genotype (PI) is also shown as ProbP in table 4. The lowest value for rank-sum nonparametric stability method (Kang, 1988) indicates maximum stability for a certain genotype. According to this statistics, genotypes Geronimo, Ebonite and Zarfam were detected as the most stable and high yield genotypes (Table 4). Kang and Pham (1991) studied several stability methods for simultaneous selection for yield and stability. They found that the rank–sum method of Kang (1988) would be useful tool for selecting simultaneously for both mean yield and yield stability that is in agreement with our result. Kaya and Taner (2003) have reported the dynamic concept of this method like to nonparametric stability measure of Fox et al. (1990). According to all nonparametric stability statistics of Thennarasu (1995), genotypes Sunday and Geronimo had the lowest of $NP_i$ and so the most stable genotypes (Table 4). These most stable genotypes, were among high mean yield genotypes. Although based on Sabaghnia et al. (2006), all nonparametric stability statistics of Thennarasu (1995) had static concept stability, but some of the most stable genotypes had high mean yield an dso reflected dynamic concept of stability. Segherloo Ebadi et al. (2008) reported that NP1 and NP4 are associated with the static or biological concept of stability. Among different nonparametric stability statistics, only Top measure and rank-sum procedure were related to the agronomic concept of yield stability (Flores et al., 1998; Sabaghnia et al., 2012). Our results are in a good agreement with the finding of the other researchers who used various nonparametric stability statistics in different crops. Traditionally, stability has been divided into distinct concepts including dynamic and static concepts (Becker, 1981). For many decades, most plant breeders used the static or biological stability concept to explain a genotype which indicates a relatively constant mean yield, independent of various environmental conditions. However, this stability concept is not acceptable to most plant researchers, who would prefer a dynamic concept of stability. In this concept of stability, it is not needed that the genotypic response to environmental conditions should be equal for all genotypes (Becker and Leon, 1988). In recent decades, most plant breeders prefer to use dynamic or agronomic concept of yield stability for GE interaction investigation and identification of the most stable genotype(s). Considering most of the nonparametric stability statistics, genotype Geronimo was the most stable genotypes. Regarding to importance of $S_i^{(1)}$ nonparametric measure some researcher use plot of mean yield of genotypes against non-parametric measure $S_i^{(1)}$ to enhance visual efficiency of genotype recommendation (Kaya and Taner, 2003). A stable genotype is one that provides high yield and consistent performance across locations (Ebadi Segherloo et al. 2008, Balalic et al. 2011 and 2013). According to this definition, only canola genotypes in section 4 can be considered as stable and with high seed yield (Figure 1).

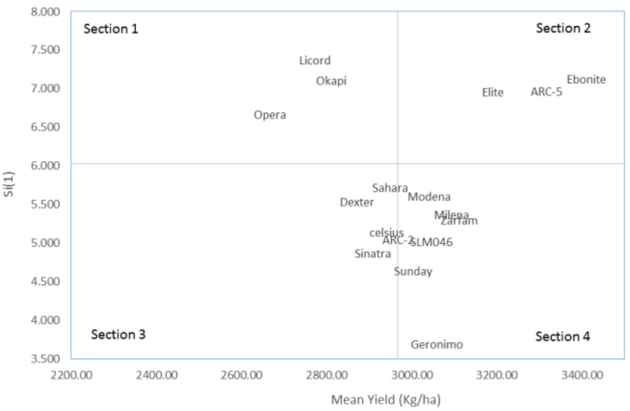

**Fig 1.** Plot of Si (1) vs. mean yield (kg $ha^{-1}$) for 18 canola genotypes over environments

### *Cluster Analysis on NPSA*

To better reveal associations among evaluated genotypes, the two-way data of genotypes' mean yield and ranks based on different nonparametric stability measures, was performed further using a clustering procedure. A hierarchical cluster analysis with squared Euclidean distance as dissimilarity measure and Ward's method revealed three major distinct groups among 17 genotypes (Figure 2). The square root of the SS difference between values for canola genotypes was used as Euclidean distance. In Ward's procedure, the dissimilarity between two clusters is shown by the "loss of information" from joining the two clusters with this loss of information measured by the increase in error sum of squares. First cluster consisted of genotypes Ebonite, Elite and ARC-5 which were high mean yield and unstable or semi-stable genotypes. Second cluster consisted of genotypes Sahara, Opera, Okapi and Licord which were low mean yield and stable or semi-stable genotypes. Third cluster consisted of all other genotypes which were moderate mean yield and moderate or stable genotypes. Although the most favorable genotype Geronimo was belong to third cluster, but it seems that this genotype as one of the most stable genotypes which had acceptable mean yield and so could be regarded for commercial release.

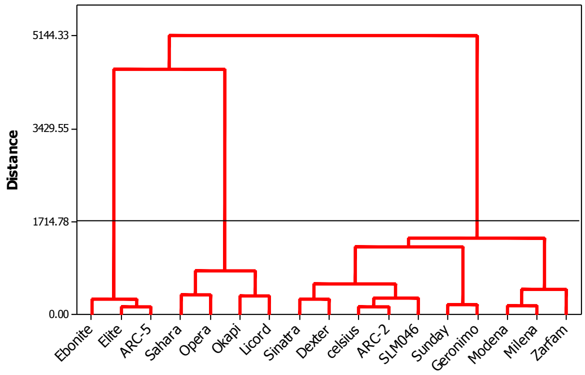

**Figure 2.** Hierarchical cluster analysis of the 17 canola genotypes based on Ward's method

### *Principal component analysis*

The level of association among stability estimates of different models is indicative of whether one or more estimates should be obtained for reliable predictions of cultivar behavior, and also could help to choose the best adjusted and most informative stability parameter(s) to fit concept of stability (Duarte and Zimmermann, 1995). Principal component analysis based on rank correlation matrices was performed to understand the relationship among the nonparametric statistics. For better visualization, the first two principal components (PC1 and PC2) were plotted against each other (Figure 3). These first two principal components explained 83% (43% and 40% by PC1 and PC2, respectively) of the variances in nonparametric stability statistics. Biplot classified the stability measures in 4 groups. The lines that connect the stability estimates to the biplot origin are called stability vectors. An acute angle between the vectors of two stability indices approximates the positive correlation between them while an obtuse angle reveals independence or very weak correlation between these stability measures. Mean yield shows a highly significant negative rank correlation with corrected mean yield. This result was also shown by Karimzadeh and his colleagues (2012). In this plot, the Low measure (LOW) and the nonparametric superiority index (PI) of Fox et al. (1990) were correlated with CV, $NP_i^{(2)}$, Rank-sum and corrected mean of rank (CMR). Sabaghnia et al. (2006) reported that the Low measure of Fox et al. (1990) and NP2 are similar in concept to GE interaction measures as it defines stability in the sense of biological concept. Top and mean rank (MR) were correlated with each other and with total mean yield. Also there is significant negative correlation between mean of yield (MY) and corrected mean of rank, Low, PI, CV, $NP_i^{(2)}$, and RS measures. However, Mahtabi et al. (2013) found significantly and negatively correlated between mean yield and $S_i^{(3)}$, $S_i^{(6)}$, $NP_i^{(2)}$ and $NP_i^{(4)}$ statistics that is in contradiction with our result. They referred the high correlation between mean yield and stability statistics to higher values of these statistics for high yielding genotypes. The non-significant and negative significant correlations between mean yield and stability parameters suggest that stability statistics provide information that cannot be gleaned from average yield (Mekbib, 2003). Kang and Pham (1991) reported that $S_i^{(3)}$ and $S_i^{(6)}$ have significant correlation with yield and stability statistics of ecovalance and deviation from regression. Meanwhile, they showed $S_i^{(6)}$ has more strongly correlated with mean yield. Positive correlation between the stability statistics $S_i^{(1)}$ and $S_i^{(2)}$ (Mohammadi et al., 2007; Scapim et al., 2000; Segherloo Ebadi et al., 2008; Zali et al., 2011) and between $S_i^{(3)}$ and $S_i^{(6)}$ (Kang and Pham, 1991; Mohammadi et al., 2007; Segherloo Ebadi et al., 2008) were reported. Our data confirm these reports in canola. Kang and Pham (1991) reported that the parameters $S_i^{(1)}$ and $S_i^{(2)}$ were nearly perfectly associated for grain yield in winter wheat. The same results, high correlations between stability parameters, are reported by Yue et al. (1997) in soybean, Scapim et al. (2000) and Delic et al. (2009) in maize, Sabaghnia et al. (2006) in lentil, Mohammadi et al. (2007) and Akcura et al. (2009) in wheat, and Segherloo Ebadi et al. (2008) in chickpea. We showed that this high correlation can be found in canola. Kaya and Taner (2003) pointed out that the method of Fox et al. (1990) is associated with the dynamic concept of stability. Also, Sabaghnia et al. (2006) and Dehghani (2008) noted that the PI nonparametric measure of stability is similar in concept to GE interaction measures as it defines stability in the sense of agronomical concept of stability.

As conclusions, producing constant yield that means yield stability and economic profitability is an important and complicated issues for plant breeders and also agronomists. The main factor behind the stability and the important aspect of any plant breeding program is GE interaction. Hence, awareness of the nature of GE interaction and yield stability is of paramount importance for canola and the other crops breeders and farmers. Successful genotypes need to be adapted to a broad range of environmental conditions in order to ensure their economic profitability everywhere. The nonparametric methods provide the opportunity of detect the nature of GE interaction. Moreover, the nonparametric method has some advantages over parametric methods. They are not limited to the assumption of normality or any distribution. They are easy to understand and the computations and interpretations are easier than those for the parametric methods. Results of different nonparametric tests for GE interactions were equivalent with conventional parametric method (combined ANOVA) in this research.

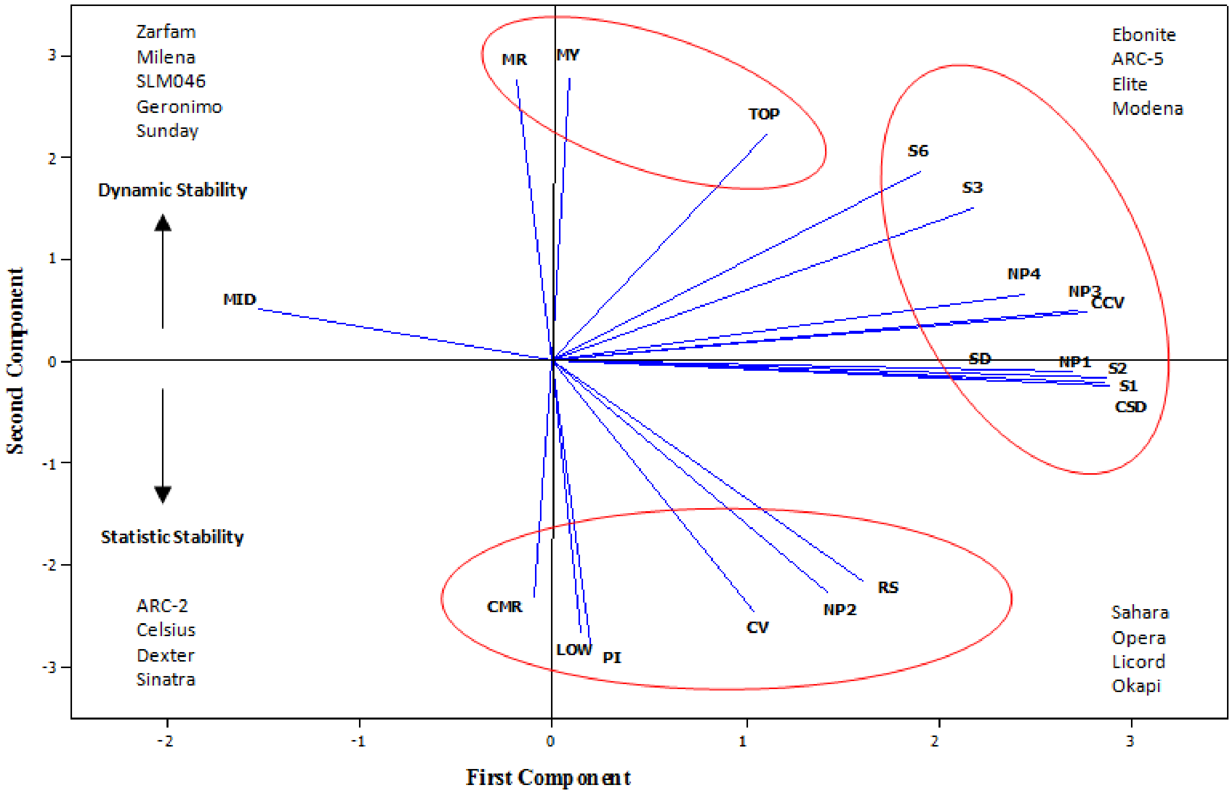

**Figure 3.** Biplot analysis of non-parametric indicators of phenotypic stability in Canola genotypes

These tests revealed existence of non-cross over GE interaction in canola genotypes multi-environment trials. The presence of significant GE interaction showed that differences among genotypes were not stable from one year to another, and these interactions are unavoidable in agricultural studies specially in multi-environment trials (Sabaghnia et al., 2010; Yan and Kang, 2003). Huehn and Leon (1995) found the same result and recommended Hildebrand and Kuninger nonparametric tests for interactions. In addition, Truberg and Huehn (2000) proposed Kroon and Laan test for cross over interaction. The genotypes used in this study did not exhibit a uniform stability and response pattern to different environments. On the basis of this assumption, genotypes with a minimal variance for yield across different environments are considered stable. Simultaneous selection for both mean yield and stability is an important consideration in breeding programs (Yan and Kang, 2003). Most plant breeders prefer simultaneous selection for mean yield and stability because the selected genotypes must have high mean values coupled with stable performance. This study showed that using corrected data on nonparametric measures of phenotypic stability is different from using original data. Similar was result found byKarimizadeh et al., 2012. They pointed out that using corrected data change Hahn's nonparametric measures nature to a static concept of stability. There is good potential in nonparametric stability methods to identify favorable genotypes in plant breeding programs. Therefore, using the agronomic or dynamic concept of stability is the better use of original dataset because the statistics of mean of ranks (MR), and Top were highly correlated with mean yield and indicated the dynamic concept of stability. The PCA biplot distinguish between measures based on static (biological) concept and dynamic (agronomic) concept of stability (Sabaghnia *et al.* 2006). The statistic Fox Top measure is related to dynamic stability and other remaining measures are associated with static stability. Researchers believe nonparametric statistics related to high seed yield performance define stability with dynamic concept (Kang and Pham, 1991; Mohammadi *et al.*, 2007 and Sabaghnia *et al.*, 2006). This research revealed that four non-parametric statistics of Huehn ($S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $S_i^{(6)}$) and three Thennarasu's statistics ($NP_i^{(1)}$, $NP_i^{(3)}$ and $NP_i^{(4)}$) clustered together and they classify genotypes as stable or unstable in a similar manner. Therefore, only one of these statistics can be applied for selecting stable genotypes in a breeding program. Among them $S_i^{(1)}$ is offered because of easy to use and simple interpretation (Huehn, 1990, 1996). According to these statistics, genotypes Geronimo and Opera had the smallest ranks and regarded as the most stable genotypes. Considering nonparametric measures including PI and King's' Rank-sum measures, genotypes Ebonite, ARC-5 and Geronimo had the smallest ranks and regarded as the most stable genotypes with high seed yield. According to statistics of Mean yield, Mean of rank and Top measures, genotypes Licord, Opera and Okapi had the smallest ranks and regarded as the most stable genotypes. Top and Rank-sum measures were positively correlated with high seed yield ($p<0.01$). Therefore, these could be recommended as useful measures for cultivar selection. Positive correlation between Rank-sum and seed yield was also reported by Zali et al, in 2011. According to the most of the nonparametric stability statistics genotype Geronimo was the most stable genotype which had relatively high mean yield. In conclusion, this genotype is ideal candidate in this regard as they had the desirable characteristics of high stability with high yield for all test environments or similar regions. Study on the association among nonparametric stability statistics is essential to make any recommendations (Dehghani, 2008; Karimzadeh et al., 2013). Among nonparametric measures, Top, $S_i^{(1)}$ and Rank-sum statistics of nonparametric procedures were found to be useful in detecting the stability of the genotypes.

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