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Comparison of Stability Parameters for the Detection of Stable and High Essential Oil Yielding Landraces of *Rosa damascena* **Mill.**

Bayzid Yousefi^{1*} and Sayed Reza Tabaei Aghdaei²

¹ Associate Professor, Research Division of Natural Resources, Kurdistan Agricultural and Natural Resources Research and Education Center (AREEO), Sanandaj, I. R. Iran 2Professor, Genetic Division, Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization (AREEO), Tehran, I.R. Iran

Received: 13 July 2019 Accepted: 29 November 2019 *Corresponding author , s email: bayzidyousefi@yahoo.com

The essential oil yield stability of damask rose (*Rosa damascena* Mill.) as an important medicinal and aromatic plant in different environments has not been well documented. In order to determine appropriate stability parameters, six statistics were studied for essential oil stability of 35 *Rosa damascena* landraces in seven locations (Sanandaj, Arak, Kashan, Dezful, Stahban, Kerman, and Mashhad) and two years (2007-2008) in Iran, using a randomized complete blocks design with three replications. Significant differences $(P<0.01)$ were observed in essential oil ratio among landraces (G), locations (L), and environments (E) and in landrace \times location (GL) and landrace \times environment (GE) interactions. The positive correlation of environmental variance (S^2) , coefficient of variation (CV), and regression coefficient of yield over environments (b) with essential oil suggest that only low-yield landraces develop a similar phenotype over a range of environments and show static stability. Although there were not any stable landraces by b ($b \approx 0$) and all of the stable ones by S^2 produced very low yields, some of the adaptable ones by CV (e.g. KM1) showed high essential oil ratios and stability simultaneously. The stable landraces according to the dynamic stability concept ($b \approx 1$, Sd² or variance due to deviation from regression \approx 0) produced moderate essential oil. Superiority index (P) determined some of the highest essential oil as adaptable landraces. The stable landraces with the least variance of the years within places $(MS_{V/P})$ produced the least essential oil. It could be concluded that a genotype can demonstrates both static and dynamic stability with high essential oil content. In addition, CV, dynamic view statistics ($b \approx 1$, $Sd^2 \approx 0$), and P are proposed as desirable parameters for the evaluation of essential oil stability with different concepts in damask rose genotypes.

A **b** S $\overline{}$ **r** $\boldsymbol{\Omega}$ \bigcap $\overline{}$

Keywords: Assessment, Greenhouse, Managerial indices, Rasht County.

Abbreviation: KM: Kerman.

INTRODUCTION

A desirable landrace is one that does not only yield well in its area of initial selection but also maintains the high yielding ability over a wide range of environments within its intended area of production. The genotype \times environment interaction (GE) is a differential genotypic expression across different environments (Basford and Cooper, 1998). According to Ramagosa and Fox (1993), the GE interaction reduces the association between phenotypic and genotypic values of a genotype. This may cause promising selections from one environment to perform poorly in one and better in another environment, forcing plant breeders to examine genotypic adaptation. Plant breeders and agronomists often ignore GE interactions and usually select genotypes based on their mean performance across environments. When all the test environments fall within some defined target environment, combining yield performance with yield stability across environments has received very little attention for practical use but could be advantageous when the target environment encompasses a wide range of environmental conditions (Kang, 1993).

Yield stability is the ability of a genotype to avoid substantial fluctuations in yield over a range of environmental conditions (Heinrich *et al.,* 1983). Adaptability or stability of a landrace often relates to physiological, morphological and phenology mechanisms. The accumulation of tolerance to a number of stresses is the key to wide adaptation, and consequently, selection in multiple environments is the best way to breed stable genotypes (Ramagosa and Fox, 1993). There are two concepts of stability, 'static' and 'dynamic'. Genotypes that are buffered against environmental variations and develop a similar phenotype over a range of environments possess a 'biological' or 'static' stability. This type is seldom a desired feature of crop landraces since no response is to improve the growing conditions which would be expected. In contrast, 'agronomic' or 'dynamic' stability permits a predictable response to environments (Becker and Leon, 1988). Researchers need a statistic that provides a reliable measure of stability or consistency of performance across a range of environments. Numerous stability parameters have been developed, but their use in selecting high-yielding and stable genotypes are limited (Kang, 1993). Lin *et al*. (1986) investigated the statistical relationship between nine stability statistics and identified three types of stability:

Type 1: Stable genotype is characterized by a small variance across all environments. This type of stability is useful when the environments considered are not very diverse and is equivalent to the static concept of stability (Becker and Leon, 1988).

Type 2: A genotype is stable if its response to environments is parallel to the mean response of all genotypes in the trial. This type is equivalent to the dynamic concept of stability (Becker and Leon, 1988).

Type 3: A genotype is stable when variance due to deviation from regression (Sd²) is small (smaller deviation from the regression). This type of stability is also dynamic and the method of Eberhart and Russell (1966) can be used for its estimation.

Furthermore, Lin and Binns (1988) defined the fourth type of stability as follows: A genotype is stable when variance due to years within locations of genotype is small (smaller variance due to years within locations). They also defined a landrace performance measure or superiority index (P)*.* Lin and Binns (1988) defined P of a genotype as mean squares of the distance between a given genotype and the genotypes with a maximum response in the locations. The smaller the distance is to the genotypes with maximum yield, the smaller the value of P is and the better the genotype will be.

Damask rose (*Rosa damascena* Mill.) is widely cultivated for its essential oil, medicinal properties, and ornamental aspects in many areas of the world, e.g. Bulgaria, Turkey, India, and Iran (Tabaei-Aghdaei *et al*., 2006; Yousefi *et al*., 2009). Since there are not any natural alternatives or artificial rose essential oil, it is one of the most expensive essential oils in global markets (Baydar

and Baydar, 2004), and the worldwide demand for high-quality rose oil is expected to rise in future (Probir, 2013). The main producers of rose oil are Bulgaria, Turkey, Iran, and India (Rusanov *et al*., 2009).

Different parts of this plant, especially its flowers, are valuable in the pharmaceutical, food, and perfume industries. The volatile or essential oil of rose is used in aromatherapy as a mild antidepressant and anti-inflammatory analgesics. Besides its application in aromatic industries, some valuable characteristics of damask rose oil such as anti- HIV (Mahmood *et al.,* 1996), antibacterial (Basim and Basim, 2003) and antioxidant (Ozkan *et al*., 2004) activities have been reported recently. Considerable variations among Iranian damask rose populations have been reported for many traits such as flower yield, oil content (Tabaei-Aghdaei *et al*., 2004, 2007), and molecular markers (Pirseyedi *et al*., 2005; Babaei *et al*., 2007; Tabaei-Aghdaei *et al*., 2006). Essential oil yield is highly influenced by many genetic and environmental factors. Therefore, the assessment of the potential of genotypes in different environments (location and years), especially in countries such as Iran with high ecological variations, is an important step in breeding programs of damask rose before selecting the desirable ones.

In this study, 35 landraces of damask rose were evaluated for essential oil in 14 environments (2 years x 7 locations). The overall objectives were to determine which stability statistics or methods can be recognized as more suitable for determining stable, adaptable and high-yielding landraces and to evaluate correlations among stability statistics and essential oil yield.

MATERIALS AND METHODS

Thirty-five landraces of damask rose were evaluated for essential oil stability in seven locations (Sanandaj, Arak, Kashan, Dezful, Stahban, Kerman, and Mashhad) with different environmental conditions (Table 1 and Fig. 1) for two years (2007-2008) in Iran. The safe and uniform (about 40 cm height) annual samplings of the landraces were procured from the experimental field of Research Institute of Forests and Rangelands of Iran (RIFR) and planted in the locations in March 2004 using a Randomized Complete Block Design with three replications. Plant spacing was set at $3^m \times 3^m$ and each plot was composed of three plants. Normal cultural practices were followed as required in each location. The essential oil was extracted by hydro-distillation (HD) and solvent (diethyl ether). Fresh petals of the plants (500 g) were subjected to hydro-distillation separately for all individual landraces and years (2007-2008) for 90 min (1.5 h) using a hydro-distillation (HD) type apparatus to produce oil. The oil was dried over anhydrous sodium sulfate and weighed. The essential oil weight of each genotype was calculated from the weight of a tube containing essential oil minus the tube weight. The essential oil ratio of the individual landraces was estimated by dividing the weight of its essential oil by its initial petal weight (500 g).

A combined analysis of variance was used to estimate the mean square of landraces, environments, and landrace \times environment interactions. Landrace stability was evaluated on the basis of landrace \times location and landrace \times environment (year \times location) interactions by following the main procedures in different concepts and types of stability.

(i) **Environmental variance** (S^2) : Landraces with a smaller S^2 are more stable. S^2 is estimated as: [1] $S_1^2 = \Sigma (Y_{ij} - \overline{Y}_{i0})^2 / q - 1$

where q is the number of environments, Y_{ij} is the yield of the ith landrace in the jth environment, and \bar{Y}_{i} is the mean yield of the ith landrace in all environments.

(ii) Environmental coefficient of variation (CV): Landraces with a smaller CV are more stable (Francis and Kannenberg, 1973). CV was estimated by:

$$
[2] \quad CV_{\dot{1}} = Si / \bar{Y}_{\dot{10}} \times 100
$$

where S_i is the environmental variance root of the ith landrace and \bar{Y}_{i0} is the mean yield of the ith landrace in all environments.

(iii) Regression coefficient of yield over environmental index (b): Finlay and Wilkinson (1963) proposed that a regression coefficient approaching zero indicates stable performance. Regression coefficients approximating 1.0 indicate average stability. Regression values above 1.0 describe genotypes with increasing sensitivity to environmental change (below average stability) and greater specificity of adaptability to high-yielding environments. Regression coefficients below 1.0 provide a measure of greater resistance to environmental change (above average stability) and therefore, increasing specificity of adaptability to low-yielding environments. We used their absolute consideration of stability that described landraces with regression coefficient (b) equal to zero as stable ones. As described by Finlay and Wilkinson (1963) and Singh and Chaudhary (1977):

$$
[3] \quad \text{bi} = \frac{\sum \text{vij}}{\sum \text{ij}}
$$

where Y_{ij} is the yield of the ith landrace in the jth environment and I_j is the environmental index and we have $I_j = \overline{Y}_{oj} - \overline{Y}_{oo}$.

(iv) Dynamic concept (b and Sd2 or deviation from the regression): Eberhart and Russell (1966) considered a stable genotype to have a slope (*b* value) equal to unity and a deviation from regression (Sd²) equal to zero. Stable genotypes will be those having mean yield higher than the average yield of all the genotypes under test. As described by Eberhart and Russell (1966) and Singh and Chaudhary (1977):

$$
[4] \quad bi = \frac{\sum vijij}{\sum ij} \text{and} \quad Sd^2i = \frac{(\Sigma \sigma^2 ij)}{q-2} \text{ that} \quad \Sigma \sigma^2 ij = \frac{(\Sigma Y^2 ij - Y^2 io)}{q} - \frac{(\Sigma Y^2 ij \text{ if } ij)^2}{\sum jij^2}
$$

where q is the number of environments, $\Sigma \sigma_{ij}^2$ is the sum of squares (SS) of deviations, $(\Sigma_j Y_{ij}^2 - Y_{i0}^2/q)$ is the total SS, and $(\Sigma_j Y_{ij} I_j)^2 / (\Sigma_j I_j^2)$ is the SS of regression. The regression coefficient of genotypes (b_i) was tested via t-test with an assumed value (β =0 in Finlay and Wilkinson and β = 1 in Eberhart and Russell model) as:

$$
\begin{array}{ll} \text{[5]} & \text{t} = \frac{\text{b} - \beta}{\frac{\text{Mse}}{\Sigma \text{Ij}^2}} \end{array}
$$

where Mse is the pooled error and I_i is the environmental index.

(v) Variance due to years within locations (MSY/P): After arranging a year-location essential oil ratio table for each landrace, $MS_{Y/P}$ was estimated as:

[6]
$$
SSY/P = SS_{\text{Total}} - SS_{\text{Places}}
$$
 and $MSY/P = \frac{SSY/P}{(y-1)1}$

where MSY/P is the variance due to years within locations, and y and l are the number of years and locations, respectively.

(vi) Landrace performance measure or superiority index (P): As described by Lin and Binns (1988):

$$
\begin{bmatrix} 7 \end{bmatrix} \quad \text{Pi} = \frac{(\overline{Y}i\mathbf{j} - \overline{Y}\mathbf{j} \text{ max})^2}{2\mathbf{l}}
$$

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where \bar{Y}_{ij} is the mean yield of the ith landrace in the jth location, \bar{Y}_{jmax} is the mean yield of the landrace with the maximum yield in the jth location, and l is the number of locations.

Mean essential oil ratio of landraces were compared with the overall mean of landraces (\bar{Y}_{oo}) via t-test as:

$$
\begin{bmatrix}8\end{bmatrix}\ \ t=\frac{(\tilde Y i-\tilde Y o o)}{\frac{\sum s d i^2}{q}}
$$

where \bar{Y}_i is the mean essential oil ratio of the ith landrace, ΣSd^2 is the pooled deviations, and q is the number of environments. In order to determine the degree of associations between essential oil ratio and stability parameters, Pearson's coefficients were used.

Fig.1. The origin sites of damask rose landraces (OS1- Os13) and research locations (L1-L7) on the map of Iran.

Table1. Some ecological characteristics of the research locations.

Locations (Provinces)	Longitude Latitude (East)	(North)	Altitude (m)	Average temperature $({}^{\circ}C)$			Relative humidity	rainfall	Annual Number of freezing	Annual	Total
				T_{Min}	T_{Max}	T_{Opt}	$\binom{0}{0}$	(mm)	days	evaporation (mm)	sunny hours
Sanandaj $(Kurdistan)$ – (L1)	$47^{\circ} 00'$	35°20'	1373.4	5.4	21.4	16	47	462.4	105.8	1340	2860
Arak (Markazi) – (L2)	49 ° 46'	34°60'	1708	6.9	20.7	13.8	46	341.5	91.4	1750	2973.3
Kashan (Isfa- han – (L3)	51°27'	33°59'	982.3	12.1	26.1	14	40	138.8	43.6	2526	2906.2
Dezful $(Khuzestan)$ - (L4)	48 ° 25'	32°16'	82.9	15.8	32	16.2	48	343.8	1.6	2334	3066.1
Stahban $(Fars)$ – (L5)	53 ° 41'	28°58'	1288.3	10.9	27.7	16.8	39	293.1	33.7	2196	3370.4
Kerman $(Kerman) - (L6)$	56°58'	30°15'	1753.8	6.9	24.7	17.8	32	154.1	89.1	1800	3165.3
Mashhad (Kho- rasan $)- (L7)$	59 ° 38'	36°16'	999.2	7	21.1	14.1	55	255	90.9	1720	2887.6

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RESULTS AND DISCUSSION

Significant differences (P<0.01) were observed in essential oil ratio among landraces (G), locations (L), environments (E) and landrace \times location (GL) and landrace×environment (GE) interactions (Table 2), and stability parameters were estimated for landraces (Table 3).

The landraces of GU1, IS6, YZ2, IS5, HA1, AR1, and KZ1 showed the lowest environmental variance (S^2) and, thus, were stable for GE (landrace \times environment) interaction. The landraces of GU1, IS6, IS5, YZ2, HA1, IS8, and YZ1were stable for GL (landrace \times location) interaction for essential oil ratio (Table 5). Kempton and Fox (1997) described adaptation as yield stability in a spatial dimension. So, we can define stable landraces for landrace × location interaction as adaptable and compatible ones. The stable landraces with the $S²$ parameter produced a very low essential oil ratio (Table 3). Environmental variance (S²) was positively correlated with essential oil ratio in both environments and locations (Table 4 and Fig. 2). The significant positive correlation between $S²$ and essential oil suggests that only landraces with a lower essential content develop a similar phenotype over a range of environments and locations. Environmental variance (S2) measures "biological" or "static" stability. This type of stability is seldom a desired feature of crop cultivars since no response to improved growing conditions would be expected (Becker and Leon, 1988). Because of the lowest oil yield of the stable landraces with S², this statistic is not a suitable parameter for evaluating essential oil stability in damask rose, especially in widely varied ecological conditions like the studied areas, so it is not recommended.

The landraces of IS5, YZ2, GU1, IS6, TH1, HA1, and KZ1 showed the lowest environment coefficient of variation (CV) and they were, thus, stable for GE and the landraces of IS5, GU1, YZ2, IS6, IS8, TH1, and KM1 were stable for GL for essential oil ratio (Table 5). The stable landraces with the CV parameter produced very low (YZ2, GU1, IS6, and HA1), low (IS5, KZ1, and IS8), moderate (TH1) and high (KM1) (Table 3). The relationship between the environmental coefficient of variation (CV) and essential oil was positive and significant (P<0.01) in both environments and locations (Table 4 and Fig. 2). This shows that an increase in essential oil yield usually occurs with an increase in CV. The stable and adaptable landraces with the CV parameter produced

** and n_s : Significant at $P < 0.01$ and insignificant, respectively. $G =$ Landraces, $E =$ Environments, $GE =$ Landraces \times Environments, E (L) = Environment (Linear), GE (L) = Landraces \times Environments (Linear) and $\sum S d_i^2$ Pooled deviation from regression.

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very low to high essential oil. The presence of the landrace of KM1 with an essential oil content of higher than average among adaptable landraces directed us to the conclusion that although we know that stable genotypes with stability type I parameters (Static stability type) such as $S²$ and CV usually produce low yields because of low responses to environments, this is not an absolute rule. In other words, we can find high essential oil yielding genotypes among biologically stable genotypes such as KM1. Since landraces with a smaller CV are more stable, so we search for highyielding and stable ones. Thus, this could be possible. Given the results, especially the potential access to high essential oil and stable genotypes with CV (e.g. KM1), this parameter can be recommended as a suitable parameter to find high essential oil yields with static stability evaluating in damask rose.

There was no stable landrace (both for GE and GL) using Finlay and Wilkinson's (1963) consideration (b or regression coefficient of yield over environmental index equal to zero) for essential oil ratio. The regression coefficient of essential oil ratio over environmental index (b value) showed a significant (P<0.01) positive correlation with essential oil ratio both in environments and locations (Table 4 and Fig. 2). This result suggests that all of the studied landraces have somewhat reacted to environmental changes. The regression coefficient of yield over environmental index (b value) in Finlay and Wilkinson's (1963) consideration (b equal to zero) measures static stability and, based on the results, only the landraces with very low essential oil ratio showed a slope equal to zero and developed a similar phenotype over a range of environments. The strong positive correlation between b and the essential oil is in accordance with this result. Therefore, this static view of b (equal to zero) is not a favorite method, so it is not recommended.

The landraces TH1, CM1, YZ1, and IS3 were stable and CM1, QM1, KZ1, KS1, and HO1 were adaptable for essential oil according to Eberhart and Russell's (1966) considerations (b equal to unity, Sd² or variance due to deviation from regression equal to zero, and mean of essential oil ratio equal to or greater than the average of landraces) (Table 5). Regression coefficient (b) showed a significant correlation with essential oil ratio in both environments and locations ($r= 0.744**$) and $r= 0.697**$, respectively). Sd² also showed a positive significant correlation with essential oil ratio in environments ($r= 0.706$ ^{**}) and locations ($r= 0.447$ ^{**}) (Table 4 and Fig. 2). The stable and adaptable landraces according to Eberhart and Russell's (1966) model produced an essential oil content of the average of the landraces. Eberhart and Russell's (1966) model measures "agronomic" or "dynamic" stability, in which a genotype is stable if its response to environments is parallel to the mean response of all genotypes in the trial. Therefore, by this method, we can determine generally stable, adaptable and moderate-yielding landraces. Freeman (1973) and Bernardo (2002) have mentioned this model as the most popular method for evaluating stability in crops. This method has been used to evaluate yield stability widely in both annual and perennial plants such as *Campanula rapunculoides* (Vogler *et al.*, 1999), *Hevea brasiliensis* (Omokhafe, 2004), and *Thea* sp. (Wachira *et al.*, 2002), and flower yield of *Rosa damascena* (Yousefi *et al*., 2009). Therefore, we recommend the method of Eberhart and Russell (1966) as a useful tool to determine general essential oil yield stability and adaptability of damask rose (*Rosa damascena*) landraces.

The landraces of SM2, IS4, SM1, AK1, and BA1 with an essential oil ratio of 0.0529, 0.0446, 0.0441, 0.0437 and 0.0426%, respectively performed the best among the studied landraces (Tables 3 and 5). They also showed the lowest superiority index (Pi). So, they were adaptable landraces with high essential oil yields. The superiority index (P_i) was negatively correlated ($r=$ -0.581**) with an essential oil ratio (Table 4 and Fig. 2). This statistic suggests that high-yielding landraces, which demonstrate high yielding potential in several locations, should be considered the adaptable ones. As such, Lin and Binns'(1988) superiority index (P) is also a suitable index to identify high essential oil-yielding and adaptable landraces in damask rose.

The landraces GU1, YZ2, IS5, IS6, IS1, IL1, and QM1, which had the lowest essential oil

ratio (Table 3), showed the lowest variance of the years within places ($MS_{Y/P}$). So, they were stable (Table 5). The variance of the years within places ($MS_{Y/P}$) showed a significant positive correlation (r=0.752**) with an essential oil ratio (Table 4 and Fig. 2). Because of mixing the year effect with the effect of plant age, variance due to years within places $(MS_V)p$) is not a suitable parameter for some age-related traits such as flower yield and the traits that are strongly correlated with plant age in perennial plants, but, due to yearly mechanism of the essential oil production, we can use this index to discriminate stable essential oil-yielding damask rose genotypes. In accordance with its strong positive correlation with essential oil, the stable landraces with the lowest $MS_{Y/P}$ showed the lowest essential oil ratio. Thus, $MS_{Y/P}$ is not a favorable stability parameter either.

Fig. 2. Response to environments indices and mean essential oil ratio of 35 damask rose landraces.

**: Significant at $P < 0.01$.

In addition, the stability parameters of Francis and Kannenberg's (1973) coefficient of variation (CV), Eberhart and Russell's (1966) model, and Lin and Binns'(1988) superiority index (Pi) can be recommended as desirable parameters and methods to evaluate essential oil yield stability of damask rose landraces.

Although, Falkenhagen (1996) and Kanzler (2002) do not recommend multivariate methods, such as additive main effects and multiplicative interactions (AMMI), because of any additive advantages versus the classic stability methods in perennial plants and forest trees, it can be

Table 5. Stable (for environments) and adaptable (for locations) landraces based on the studied stability parameters for essential oil ratio.

recommended to further study the use of AMMI and also non-parametric statistics such as Kang rank sum (KRS) (Kang, 1988) and stability index (I) (Bajpai and Prabhakaran, 2000) in discriminating stable, adaptable and high essential oil landraces of *Rosa damascene*.

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