Selection criteria of flax (*Linum usitatissimum* L.) for seed yield, yield components and biochemical compositions under various planting dates and nitrogen

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This study was conducted to estimate the best applications of N levels (0 to 150 kg ha⁻¹) and 5 sowing dates, selection criteria and interrelationships of seed yield, yield components and 5 biochemical compositions of 8 linseed cultivars. The experiments were carried out at Yasouj Azad University, Iran, during 2009 and 2010 at a field experiments in RBD split-plot with 4 replications. The results of separate or combined trials indicated that the first sowing date (14th March) along with 100 and 150 kg ha⁻¹ of N produced the highest yield and yield components. Moreover, positive associations observed between seed yield with most of the traits or oil percentage and linoleic fatty acids. As expected, significant correlations observed between oil with oleic acid (r = 0.599***) and protein (r = -0.794***) percents. Path analysis in more extent revealed positive direct effects of almost all the traits on yield. Capsule number and primary branch per plant, plant height and 1000-seed weight had the most positive direct effects on seed yield. Biochemically, oleic and linoleic acids had the most effects on seed yield. Conformingly, the principal component analysis (PCA) showed that capsule number and primary branch per plant, plant height and 1000-seed weight, oleic and linoleic acids along with dry weight had the most contributions, interpreting almost all the variation of traits. Indeed, to simultaneously breed of high yielding and oil flax varieties, capsule number per plant, primary branch per plant, plant height and 1000-seed weight should firstly be considered followed by oleic and linoleic fatty acids.

**Key words:** Flax (Linseed), path coefficient analysis, principal component analysis, yield components.

INTRODUCTION

Flax (*Linum usitatissimum* L.; n = 15), also called linseed, an important oilseed crop belonging to Linaceae family, with 14 genera and over 200 species, is the only species in this family with economic and agronomic values (Tadesse et al., 2009). It has nutrients and pharmaceutical uses and used for edible and lightening purposes in some Asian countries and can also be used as a substitute part of animal fat in poultry diets (Khan et al., 2010). Possibly, this crop species has originated from *Linum angustifolium* Huds. (n = 15), native to the Mediterranean region (Tadesse et al., 2009). The cultivated *Linum* species have more economic values. It is an annual and rarely perennial (plant) with 2 different forms, used for fiber and oil production (Kurt, 1996a). Almost all the species are annual herbs and some are shrubs, *L. usitatissimum* L. is the only species with non-dehiscent capsules suitable for modern cultivation of the family Linaceae (Alemaw and Alemayehu, 1997). Flax seeds contain 30 to 45% oil, making it an important industrial crop. Due to rapid drying off, flax oil is quite valuable in dye industry. The linseed contains about 36 to
48% oil content which is high in unsaturated fatty acids, especially linolenic acid (Enser et al., 2000; Kouba et al., 2003; Kouba, 2006; Khan et al., 2010).

Selection is a pivotal axis of breeding programmes by which genotypes with high productivity in a given environment may be developed. The yield of flax is related to its components as number of plant per unit area, number of capsules per plant and weight of seeds per capsule and so on. The main target of improvement programmes is achieving varieties having high yield and quality. Obtaining high yielding and high quality linseed varieties depend upon applying fertilizers like N. Historical (successive/evolutionary) increases in cereal yield have depended on large inputs of N fertilizer (Makino, 2011). For instance in rice, since single seed weight is genetically constant irrespective of N application and growth environments (Yoshida, 1981), yield is simply determined by the product of the two elements: Seed number and the ratio of filled seeds, both of which are affected by N application. In other words, seed yield is determined (multiplied) by the product of seed number and intact seeds which are influenced by N application denoting the importance of N fertilizer.

Selection for yield has many complexities because it is end product of various yield components or yield contributing characters, which are naturally polygenic inherited and majorly influenced by environment. So due to less impression of direct selection for yield, more efforts should be over indirect selection for yield components (changing yield through yield components). Proper understanding of association of different traits, provide more reliable selection criterion to achieve a high seed yield (Akbar et al., 2001). This selection criterion takes into account the information on interrelationships among agronomic characters, their relationship with seed yield as well as their direct influence on seed yield. However, simple correlation coefficients and ANOVA tables (even in multiple-year trials) may not evolve satisfactory results in uncovering the real interrelationships among agro-biochemical characters. Nevertheless, selection for yield via highly correlated characters becomes easy if the contribution of different characters to yield is quantified using path coefficient analysis (Dewey and Lu, 1959). In fact, practically selection criteria will contribute to selection based on direct effects (Yucel, 2004; Copur and Oglakci, 1998). So path coefficient analysis technique is a statistical approach which is based on multiple regression and is useful for revealing the direct and indirect effects of characters in a network of factors like agro/morpho/physio/biochemical traits which is able to separate correlation coefficients into their components of direct and indirect effects (Dewey and Lu, 1959; Wright, 1960). However, achieving the interrelationships among characters aids in programming efficient approaches of multiple trait selection.

In the case of flax, positive relationships between seed yield, number of capsules, number of primary branch and plant height are reported (Basu and Bose, 1976; Can et al., 2001, 2003; Kaynak, 1998; Vijayakumar et al., 1976). Moreover, significant direct effects of 1000-seed weight, number of capsules per plant and seed number per capsule have been previously reported (Vijayakumar and Rao, 1975). It is reported that the strongest factors influencing seed yield have been: Plant height, seed number per capsule, capsule number per plant, 1000-seed weight and primary branch number, respectively (Nie et al., 1995). Considering yield attributes, many researchers (Patil et al., 1989; Musuli and Patnaik, 1994; Mishra and Singh, 1992; Khan et al., 1998; Agrawal et al., 1994) studied magnitude of direct and indirect effects, through path coefficient analysis.

The present study was conducted to study and determine main possible associations among yield, yield related and some biochemical characters (protein, oil and fatty acids compositions) in linseed under different nitrogen and planting dates to determine the best selection criteria to enhance seed yield of flax.

MATERIALS AND METHODS

Plant materials

The experimental entries were 8 flax (linseed) varieties including Atlante, Bionda, Raulinus, Astamm2.764, Somaco, Linda, Olay-ozon and Saidabad.

Experimental design

The study pertaining to assessment of selection criteria in oil flax using correlation coefficients, regression analyses, path analysis and principal component analysis conducted in the Department of Agronomy, Yasouj Azad University, during spring, years of 2008 and 2009 and 2009 to 2010 located at 30˚ 50’ E latitude, 51˚ 41’ longitude with altitude of 1831 m above sea level. Experimental design was a split-plot in 4 replication randomized block design combined for over 2 years. Main and sub plots separated 1 and 0.5 m, respectively. The experiments were conducted on clay loam soil, irrigation and other cultivation management (fertilizers, weeding etc.) was well done till harvest of plants. The flax seeds cultivated with 5.7×105 plants per hectare. Each experimental plot was provided 5×2 m2 plot size and consisted of four 4 m-long rows with inter-row spacing of 25 cm and inter-plant spacing of 7 cm. Moreover, fertilizers were applied at the rate of 90/80 kg/ha-1 N/P2O5. Amount of seeds oil was measured using Soxhlet apparatus (Soxhlet, 1879; Dorina et al., 2008), and fatty oleic, linoleic and linolenic acids extracted using gas chromatography (GC). In addition to oil and fatty acids, seed yield (kg/ha-1), harvest index (HI), 1000-seed weight (g), plant height (PH), primary branches number per plant, capsules number per plant, and dry weight (biomass, g) were recorded following to harvest. Then mean of 10 samples were used for statistical analyses.

Statistical analyses

The collected data were analyzed by ANOVA to determine significant differences between the 8 genotypes using SAS (Ver. 9.1, SAS Institute Inc., Cary, NC) and Minitab (version 15 to 16, LEAD Technologies, Inc, USA). ANOVA for all the traits was
Table 1. Phenotypic correlation coefficients between various characters in oil flax.

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Seed yield (kg ha(^{-1}))</th>
<th>HI</th>
<th>1000W</th>
<th>PH</th>
<th>P.branch</th>
<th>Caps.no</th>
<th>Oil (%)</th>
<th>Prot. (%)</th>
<th>Dry-wt</th>
<th>Linolenic</th>
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<tbody>
<tr>
<td>HI</td>
<td>0.39*</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>1000W</td>
<td>0.90**</td>
<td></td>
<td>0.94**</td>
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<tr>
<td>PH</td>
<td>0.93**</td>
<td>0.52* 0.566*</td>
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<tr>
<td>P. branch</td>
<td>0.85**</td>
<td>0.65** 0.72** 0.91**</td>
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<tr>
<td>Caps.no</td>
<td>0.98**</td>
<td>0.74** 0.79** 0.93** 0.938**</td>
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<tr>
<td>Oil (%)</td>
<td>0.81**</td>
<td>0.80** 0.84** 0.47<em>ns 0.27</em>ns 0.37*ns</td>
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<td>Prot. (%)</td>
<td>-0.34*ns</td>
<td>-0.41<em>ns -0.41</em>ns 0.44** 0.202<em>ns 0.11</em>ns -0.79**</td>
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<tr>
<td>Dry-wt</td>
<td>0.42*ns</td>
<td>0.65** 0.69** 0.96** 0.95** 0.98<em>ns 0.23</em>ns 0.25*ns</td>
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<tr>
<td>Linolenic</td>
<td>-0.33*ns</td>
<td>0.41<em>ns 0.46</em> 0.81** 0.75** 0.74** 0.43<em>ns 0.50</em> 0.77**</td>
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<tr>
<td>Oleic</td>
<td>-0.73*</td>
<td>0.20<em>ns 0.13** -0.70** -0.45</em> -0.46* 0.60** -0.81** -0.56* 0.56*</td>
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<tr>
<td>Linoleic</td>
<td>0.81**</td>
<td>0.56** 0.60** 0.95** 0.90** 0.93** 0.12<em>ns 0.41</em>ns 0.95** 0.89**</td>
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</table>

Ns: Non-significant (P>0.05); *, significant (P<0.05); **, significant (P<0.01). HI, harvest index; 1000W, 1000-seed weight; PH, plant height; P.branch, primary branch; Caps.no, capsule number; Prot, protein%; Dry-wt, dry weight.

Correlation studies

The possible enhancements of high yield through yield attributes, as primary target of crop improvement, requires understanding the amount of correlations among various yield contributing characters or in fact, yield components. The simple correlation coefficients between quantitative characters in this experiment combined for over 2 years is given in Table 1.

Association of seed yield with other traits combined for over 2 years

According to Table 1, there were positive significant correlations between seed yield with HI, 1000-seed weight, plant height (PH), primary branch per plant, and capsule number per plant, oil and linoleic acids percentages. Similar results reported (Akbar et al., 2001; Mirza et al., 1996; Tadesse et al., 2009) show positive and significant relationships of HI and capsules per plant with seed yield observed. This confirms that such characters could be properly applied to improving yield through selection. Of course, seed yield (kg ha\(^{-1}\)) showed negative and non-significant associations with protein and linolenic acid contents, but a positive and non-significant correlation with dry weight. On the other hand, oil and linoleic acid contents exerted low to moderated negative direct effects on seed yield (Table 2), whereas they exhibited positive associations with it. As aforementioned, harvest index and number of capsules per plant showed positive and significant associations with seed yield, confirming earlier (Tadesse et al., 2009; Mahto and Mahto, 1997) reports. Though, the associations of protein, dry weight and linoleic acids with yield were non-significant, the positive associations of
such traits indicated especially that such characters could be utilized for indirect selection in yield breeding programmes.

**Associations of oil content (%) with seed yield and other traits**

As previously mentioned, phenotypic correlation coefficients for over 2 years (combined) showed (Table 1) highly significant associations between percent oil content with seed yield, HI, 1000-seed weight, oleic acid percentage (r = 0.60**) and negatively significant with protein content (r = -0.79**). The associations of oil with dry weight, linoleic and linolenic contents, plant height, primary branch and capsule number per plant were positive but non-significant. Some of these findings for oil associations were similarly reported by Tadesse et al. (2009) who indicated non-significant and positive associations of oil content with number of capsules and seed yield per plant. According to Tadesse et al. (2009), other studies (McGregor, 1936; Johnson, 1932; Geddes and Lehberg, 1936; Chu and Culbertson, 1952) reported a positive association of oil content with seed weight in flax. Moreover, Comstock (1960) reported a positive correlation between the seed weight and percent oil content of the individual F$_2$ and F$_3$ plants from two crosses. This suggests that one can select simultaneously for seed yield as well as high oil content by selecting for seed weight (Tadesse et al., 2009). As pointed out, flaxseed is used for oil production, rich in essential poly unsaturated fatty acids such as alpha-linolenic acid and rich supply of soluble dietary fiber. Flaxseed oil is also used as an industrial drying oil due to its high linolenic acid content (Muir and Westcott, 2003; Green, 1986). However, some flax genotypes have been developed which contain very low levels of linolenic acid in their oil, making them suitable for use as edible-oil (Green, 1986; Rowland, 1991). As shown in Table 1, linseed percent oil content in this experiment in separate years (data not shown) and (combined) showed positive highly significant associations with seed yield (Figure 1), 1000-seed weight and oleic acid percentage. The linear regressions of seed yield with other characters in flax in current experiment (Figure 1) suggest an approach for accuracy of decision simultaneous selection of seed yield and other traits. Indeed, this implies that genotypes with high oil contents may have higher grain yield, higher seed weights, and higher oleic acid content. These characters could be of great value and may be conducted as selection criteria for obtaining entries with high yield with higher oil, oleic acid and weight. This suggests possibility of simultaneously improvement of seed yield as well as oil content, as Tadesse et al. (2009) implied. The importance of such characters on seed yield and simultaneous selection of them are discussed later through principal component analysis.

**Path coefficients analysis**

Correlation coefficient measures the mutual association between two variables but does not permit the cause and effect relationship of traits contributing directly or indirectly towards economic yield. Whereas, the path coefficient analysis specifies the causes and measures their relative importance (Shivanna et al., 2007). This kind of analysis is of a more precise way than simple correlation coefficients by which partitioning the correlations into direct and indirect effects of individual traits (independent) on final dependent trait (grain yield) is achieved. Such an analysis helps the breeders to identify the characters that could be used as selection criteria in linseed breeding programmes. In current experiment, the phenotypic correlations were further analyzed by path coefficients analysis, which decomposes the correlation coefficients into their related direct and indirect effects through specific pathways called
Figure 1. Association between seed yield and agronomic and biochemical traits in linseed of 8 oil linseed. HI, harvest index; 1000 W, 1000-seed weight; PH, plant height; P. branch, primary branch; Caps. no, capsule number; Prot, protein%; Dry-wt, dry weight.

Figure 2. Path analysis diagram representing direct effects and their significance of agro-biochemical traits on seed yield of oil linseed. P. branch: Primary branch per plant; 1000 w: 1000-seed weight; PH: Plant height; Caps. no: Capsule number per plant; HI: Harvest index.

Path coefficient analysis of agronomic and biochemical traits of linseed on seed yield demonstrated (Table 2 and Figure 2) that capsule number per plant (0.93**), primary
Table 3. Eigenvectors and eigenvalues of 4 principal components for 12 morphological and biochemical characters of 8 linseed accessions tested at 2 years.

<table>
<thead>
<tr>
<th>Variable component</th>
<th>Yield</th>
<th>Hl</th>
<th>1000W</th>
<th>PH</th>
<th>P.branch</th>
<th>Caps.no</th>
<th>Oil%</th>
<th>Protein%</th>
<th>Dry-wt</th>
<th>Percent of fatty acid</th>
<th>Percent of fatty acid</th>
<th>Percent of fatty acid</th>
<th>Percent of fatty acid</th>
<th>Eigenvalue</th>
<th>Proportion</th>
<th>Cumulative</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC1</td>
<td>0.33</td>
<td>0.01</td>
<td>0.32</td>
<td>0.33</td>
<td>0.3</td>
<td>0.33</td>
<td>0.33</td>
<td>-0.24</td>
<td>0.33</td>
<td>0.13</td>
<td>0.3</td>
<td>0.31</td>
<td>0.31</td>
<td>8.45</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>PC2</td>
<td>-0.12</td>
<td>-0.69</td>
<td>0.13</td>
<td>0.03</td>
<td>-0.16</td>
<td>-0.03</td>
<td>-0.45</td>
<td>-0.12</td>
<td>-0.28</td>
<td>-0.21</td>
<td>-0.06</td>
<td>1.71</td>
<td>0.14</td>
<td>0.85</td>
<td>0.14</td>
<td>0.85</td>
</tr>
<tr>
<td>PC3</td>
<td>0.13</td>
<td>-0.34</td>
<td>0.07</td>
<td>-0.18</td>
<td>0.07</td>
<td>0.18</td>
<td>0.06</td>
<td>-0.13</td>
<td>0.8</td>
<td>-0.05</td>
<td>-0.37</td>
<td>1.14</td>
<td>0.10</td>
<td>0.94</td>
<td>0.10</td>
<td>0.94</td>
</tr>
<tr>
<td>PC4</td>
<td>-0.17</td>
<td>-0.41</td>
<td>-0.27</td>
<td>-0.22</td>
<td>0.07</td>
<td>-0.21</td>
<td>-0.08</td>
<td>0.29</td>
<td>0.28</td>
<td>0.04</td>
<td>0.63</td>
<td>0.25</td>
<td>0.31</td>
<td>0.31</td>
<td>0.03</td>
<td>0.97</td>
</tr>
</tbody>
</table>

HI, Harvest index; 1000 W, 1000-seed weight; PH, plant height; P.branch, primary branch; Caps.no, capsule number; Prot, protein%; Dry-wt, dry weight.

branch per plant (0.70**, plant height (0.44**), 1000-seed weight (0.40*) and HI (0.12*), had the most significant direct effects on seed yield, followed by fatty acids and protein content, respectively. These traits had also the most correlation coefficients with seed yield. In this subject, other traits such as oleic and linoleic percents though had high correlations with seed yield but had non-significant direct effects on seed yield. Moreover, dry weight and linolenic acid percents had not only significant associations, but non-significant direct effects on grain yield. Such results are reported by Tadesse et al. (2009) where thousand seed weight and oil content had significant positive correlation with yield per plot but their direct positive effects were low. This experiment indicated that characters such as capsule number per plant, primary branch, plant height and 1000-seed weigh were the most contributors to seed yield. Hence these traits could be accounted and explored more confidently for indirect selection (selection criteria) for yield improvement due to their good impacts on linseed yield.

It is also generally accepted that in path coefficient analyses, where a given direct effect (s) is low, its attributed indirect (s) effect through other variables is high or vice versa. For instance, capsule number, which had a high direct effect (0.926**) and subsequent low indirect effects (Table 2). In parallel, it was observed that the highest indirect contributions mainly exhibited three notable characters, that is, by capsule number per plant, plant height and 1000-seed weight, followed by fatty acid compositions. Therefore, such traits should be given emphasis as suggested by Ali et al. (2009) in selection process. These results concluded that improvement of seed yield in oil linseed is linked with these traits. And as Ali et al. (2009) suggested, these parameters should be an integral part of effective selection criteria leading to yield enhancement in linseed. In addition, according to Shivanna et al. (2007) the knowledge of correlation and path coefficients analysis to visualize the genetic association among various traits will be useful in selecting diverse genotypes to be included in hybrid breeding programmes to exploit greater heterosis.

Principal component analysis of seed yield and other traits

The principal component analysis (PCA) was performed for morphological and biochemical traits (Table 3 and Figure 3) which revealed the four most informative principal components with eigenvalues of 8.45, 1.71, 1.14 and 0.31, respectively, which together accounted almost for the entire variability. The first 4 eigenvectors of all characters explained 97% of the total variance. Thus, according to PC1, characters such as seed yield, plant height, capsule number, oil and linolenic acid contents had relatively higher contributions to the total morphological variability, a truth which proved by other statistical approaches similar to path coefficients analysis which discussed at earlier parts of this paper. Other characters, that is, primary branch number, 1000-seed weight, linoleic content, dry-weight and protein content exerted the most variability in this trend.

The first two principal components biplot (Figure 3) including loadings of the various characters along with the genotypes spread over is given in Figure 3. This Figure indicates that the 8 genotypes in this experiment could be categorized at 3 groups. So that, one of the groups comprising of Linda, Olay-ozon and Saidaabad had the higher protein contents, the second group including varieties Somaco and Astamm2.764 contained higher primary branch per plant. The 3rd group had higher values for the rest characters. PCA showed a clear differentiation between flax cultivars from each others. The majority of the variation (70%) is explained by
the first two principal components and in total this biplot explained 85% of the variation. Thus, this biplot can interpret the near-real differentiation of the linseed cultivars and morphological and biochemical characters studied in this experiment. This is an illustrative and fascinating result which is likely unique in linseed experiments.

Vromans (2006) examined 110 flax accessions and a wild species from different locations such as Dutch, French and Canada countries to investigate the hereditary basis of important traits such as disease resistance and fiber quality using molecular markers. Of course, he achieved a biplot of two first principal components together which explained approximately 22% of the variation. We here observed as could given in the Figure 3 that all the linseed varieties in this experiment are differentiated in 3 groups where Saidabad, Olay-ozon and Linda varieties are included in the first group and Atlante, Bionda, and Raulinus in the 2nd group and finally Somaco and Astamm2.764 in the 3rd group. As Vromans (2006) pointed out, the PCA indicated a clear differentiation and also high inter-population distance supported the differentiation between the two morphological groups. According to the biplot, for instance as mentioned earlier, cultivars Saidabad and Olay-ozon had more protein contents and so on. Thus this biplot could be used as a vital instrument to categorize, differentiate and address the genetic entities in breeding decisions.

Conclusion

The selection criteria was conducted to evaluate interrelationships of seed yield, yield components/contributors and 5 biochemical compositions of oil linseed (**L. usitatissimum* L.) in a two-year (2009 to 2010) study. The result of ANOVA showed significant differences between genotypes, N and planting date levels. By this, N levels and planting dates had severe influences on the agronomic and biochemical compositions of linseed which could be utilized in programming breeding and field trials, so that by the selection of a best combination of N fertilizer levels and sowing dates, a proper/higher yield and quality for flax will be achieved. Moreover, there were positive significant associations between seed yield with most of the characters examined. The positive associations of such traits indicated especially that such characters could be applied for indirect selection in yield breeding programmes. Moreover the associations of biochemical compositions suggest the possibility of simultaneously improvement of seed yield, as well as
oil content. As discussed, this experiment was conducted to evaluate the effects of agronomic and biochemical traits using regression fitting, path analysis and principal component analyses by them most informative characters (capsule number, primary branch, plant height and 1000-seed weight) on flax seed yield were satisfactorily uncovered. Additionally, the PCA showed that the first two components explained most (85%) of the existing variation by its associated biplot genotypes and loadings of the characters drawn which led to an informative tool to evaluate the genotypes and associations of characters as a guide for flax breeding schemes.

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