ESTIMATION OF YIELD STABILITY FOR FLAX GENETIC RESOURCE USING REGRESSION AND CLUSTER ANALYSIS

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Abstract
Flax (*Linum usitatissimum* L.) fibre and seeds are widely used to produce healthy and environmentally friendly products. It is known that the main factors that influence flax fibre' and seeds' yield are genotype and growing conditions. The main task of the Latvian flax breeding program is to develop flax genotypes that are highly productive (both fibre and seeds) and well adaptable to changing environmental conditions. Goal of this study is to identify high yielding genotypes with good adaptation to local variable agro-ecological conditions. The agronomically important traits, such as yield of stem and seeds, total and technical plant height and fibre content were evaluated for 13 Latvian origin flax lines and standard variety 'Vega 2'. The field trials have been carried out over the period from 2012 to 2015 at the Agricultural Scientific Centre of Latgale. The regression, correlation, coefficient of variation and cluster analysis between yield and yield provided components were used for identification of high yielding genotypes with good adaptation. On the basis of cluster analysis the genotypes were classified in two groups by lower and higher yield of stem, fibre content, total plant height and technical plant height. The line 'I18-1' was identifying as genotype with the highest average yield of stem (751.25 g m^{-2}) as well as highest yield against other genotypes in moisture and drought years. Most valuable by the average technical plant height was line 'L26-1' (73.05 cm).
Key words: flax, genetic resource, adaptation.

Introduction

Flax (*Linum usitatissimum* L.) is an important industrial crop with ancient farming history, but only in the last century breeding for fibre use (fibre flax) or seed use (linseed flax) has resulted in two plant types which differ considerably in morphology, anatomy, physiology and agronomic performance (Diederichsen & Ulrich, 2009). Compared to oilseed cultivars, fibre flax is typically taller, with less branching and lower seed production (Booth *et al*., 2004). The flax fibre is soft and flexible, and it is stronger than cotton or wool fibre. The flax fibre is used for manufacture of textiles, raw flax fibre is also used to make high quality paper and components for the motor industry (Berger, 1969; Bakry *et al*., 2014). Production of flax fibres left over huge number of by-products such as the cuticle, shiver and fibre fragments that currently increase interest in production microcrystalline cellulose from plants and their potential as value-added products ranging from biocomposites for medical devices to solidified liquid crystals (Akin, 2013).

Flax is highly self-pollinating, with outcrossing rates from 0.3 to 2% under normal circumstances (Booker *et al*., 2014). Taking into account the facultative cross-pollination, the development of genetically stable lines could take more than 15 years (Rashal & Stramkale, 1998). Major breeding objectives of Latvia's flax breeding are to create early or mid-early ripening varieties of flax with the improved yield (seed or fibre) and oil content, high fibre quality, resistance to lodging diseases (Grauda, Stramkale, & Rashal, 2004). Flax requires abundant moisture and cool weather during the growing season (Berger, 1969; Bakry *et al*., 2014). The cultivation techniques, weather and soil conditions as well as the flax stem processing (scratching, hackling) have a great impact on fibre quality (Grashchenko, 1963; Karpunin, 1995; Polonetskaya, Panifedova, & Sakovich, 2001; Wretfors, 2005). Harvesting of flax on the early stage of yellow ripeness allows to obtain fibres of higher quality. Flax early sowing significantly increases fibre bundles and the number of elements of fibre in the bundles. Technical plant height is characterized by the most valuable part of stem from which the long fibre is obtained (Ivanovs & Stramkale, 2001).

The ability to develop high yielding stable cultivars is a primary focus in most breeding programs and is ultimately more important than the identification of unstable cultivars. Understanding of the environmental responses of flax lines is fundamental to improving efficiency of flax production. Cluster analysis is widely used in agriculture to data processing for different crops as rapeseeds (Rameeh, 2015), barley (Fotakian *et al*., 2014), as well as flax (Bakry *et al*., 2014). Cluster analysis divides data into clusters. That is important to the strategy of classifying variability on a large number of varieties, or to reveal the genetic diversity among varieties and their response to the environmental conditions. In this way, cluster analysis is a suitable solution to group and select desirable genotypes. The correlation, regression analysis and coefficient of variation were used to determine yield stability and ecological plasticity in changeable environmental conditions (Mustăţea *et al*., 2009;

Kazmi & Rasul, 2012). Objectives of this study are evolution and investigation of Latvian fibre flax genetic resources' diversity and identification of high yielding genotypes suitable for cultivation in local variable agro-ecological conditions.

Materials and Methods

Field experiments

The field trials were conducted over the period from 2012 to 2015 at the Agricultural Scientific Centre of Latgale. Experimental material for present study consisted of 13 fiber flax lines as well standard variety 'Vega 2' was used. Plants were grown in random block plots 2 m^2 with a distance between rows 10 cm, 1700 flax seeds per 1 m^2 were sown by hand with sowing depth 1.5-2 cm in three replications. Prior to that sowing seeds' germination tests were performed for all used genotypes. Experimental plots were separated by one meter wide buffer zones. Seeds were sown between 1st and 2nd decades of May.

Flax was grown in humi-podzolic gley soil. The main agrochemical parameters of the arable soil layer were following: humus content – 6.5%, pH_{KCl} – 6.4-7.0, available P_2O_5 – 130-145 mg kg^{-1} and available K_2O – 118-124 mg kg^{-1} soil (by results of State Plant Protection Service). Complex fertilizer NPK 16:16:16 - 300 kg ha^{-1} was applied after first soil cultivation. For plants' further development a surface fertilizer – ammonium nitrate 30 kg N ha^{-1} in "fir tree" phase was applied. Herbicides ('Glins' 10 g ha^{-1} and 'Kemivets' 200 ml ha^{-1}) were used to control weeds and insecticides ('Fastac 50' 0.4 l ha^{-1}) sprayed against flax flea beetles as it is required by instruction. Tractor-drawn sprayer 'Pilmet 412' was used.

Evaluated flax varieties and lines

'S29-1', 'S29-2', 'S37-1', 'S37-2', 'T36-1', 'T36-2', 'T36-3', 'K9-1', 'K9-2', 'L26-1', 'I7-1', 'I7-2', 'I18-1' (Agricultural Scientific Centre of Latgale origin lines) and 'Vega 2'(ST) (Lithuania origin variety). Agriculturally important traits were determined such as flax total plant height, technical plant height, fibre contents, yield of stems and seeds. The genotypes were determined in phenological stages in each plot. The total and technical plant heights, fibre content were determined using randomly selected most typical 20 plants in each parcel area before the harvest. Plants were pulled manually at the stage of early yellow ripeness and then left on ground for air-drying for 5-8 days. The seedpods were removed by 'Eddi' device. Seeds were cleaned with 'MLN' sample cleaner. The yield of seed was weighed and then re-calculated to weight by 100% purity and 12% humidity. The yields of stem and seed were determined in each harvested parcel area (according by Cabinet Regulation No. 518/ 2012 Regulations for the Assessment of Value for Cultivation and Use of Plant Variety).

Meteorological conditions

Agro-meteorological conditions were determined by using ADCON – installed meteorological stations that were connected to the computer program Dacom Plant Plus. The facility provided information in direct nearby field trials. The amount of precipitation in 2013 growing period was by 22% lower and in 2015 by 6% lower in comparison to the long-term average of 311 mm (by 1. Fig.). However, precipitation in 2012 was by 50% and in 2014 by 14% higher than the long-term average. According to the air temperature,

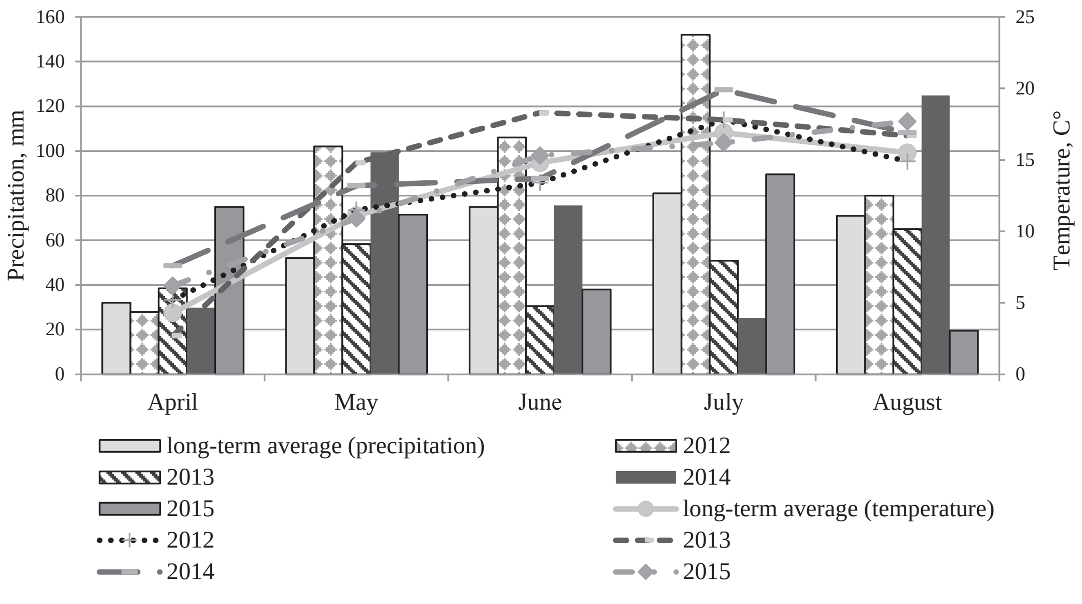

Figure 1. 2012 – 2015 sum of precipitation (mm) and average of air temperature (°C) value in vegetation period of flax.

four growing periods were warmer, than generally in long-term average. In 2012 average air temperature was 12.56 °C in 2013, it was 14.06 °C, in 2014 and 2015 it was 14.26 °C and 13.26 °C respectively, while the long-term average result is 12.52 °C.

Data analysis

MS-Excel and SPSS software were used for data statistical analysis (Arhipova & Bāliņa, 2006). Data analysis tools provide descriptive statistics, coefficient of variation (CV), regression, correlation and cluster analysis. Correlation analysis finds out significant or insignificant relationships between precipitation and yield of stem and seeds for each genotype. Regression analysis finds out significant or insignificant relationships between total plant height and sum of precipitation using linear regression for each flax variety and lines by MS-Excel software. Coefficient of variation of yield of stem was displayed against average yield of stem identifying yield stability of genotypes. Cluster analysis was used to construct a distance matrix using the Euclidian coefficient and based on the Ward method. Cluster analyses included flax yield of stem, fibre contents, total plant height and technical plant height. Before computing the distance between varieties, our data were standardized as recommended by Ward (1963).

Results and Discussion

According to the data, the amount of precipitation during the growing season plays a major role in flax yield of stem (Fig. 2.).

The largest yield of stem increase was observed in 2012. Evaluating the period in 2012 from germination until full flowering of flax, it was characterized by high moisture content (102 – 152 mm) from May to July that favorably increased flax stem growth. The drought in April 2012 did not have a significant negative impact on the germination and plant development. In 2014, the lowest stem yield increase was obtained.

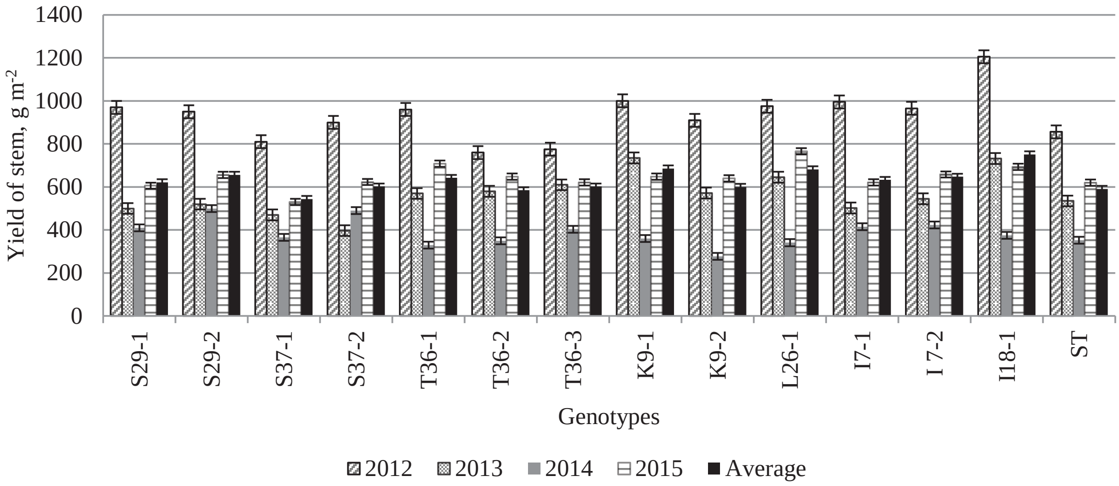

Figure 2. 2012 – 2015 yield of stem (g m^{-2}) of lines and variety of flax.

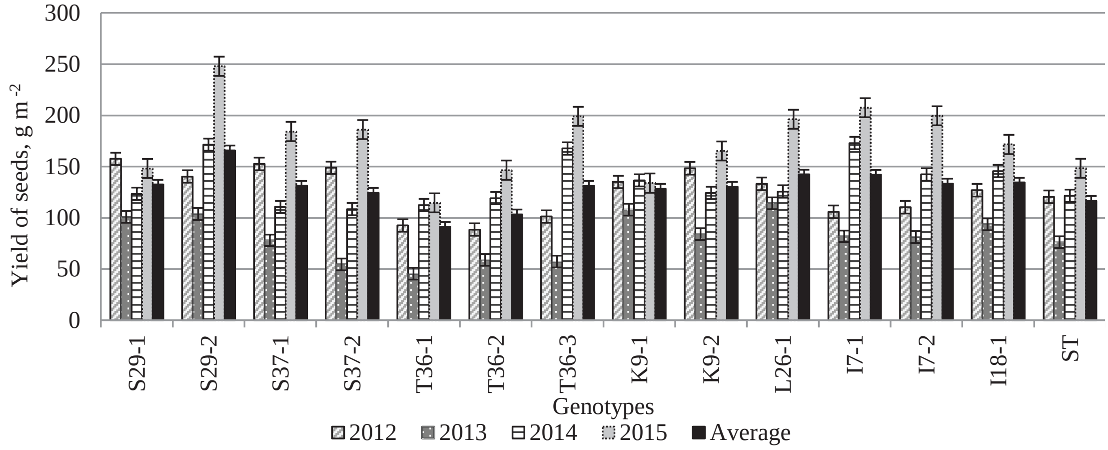

Figure 3. 2012 – 2015 yield of seeds (g m^{-2}) of lines and variety of flax.

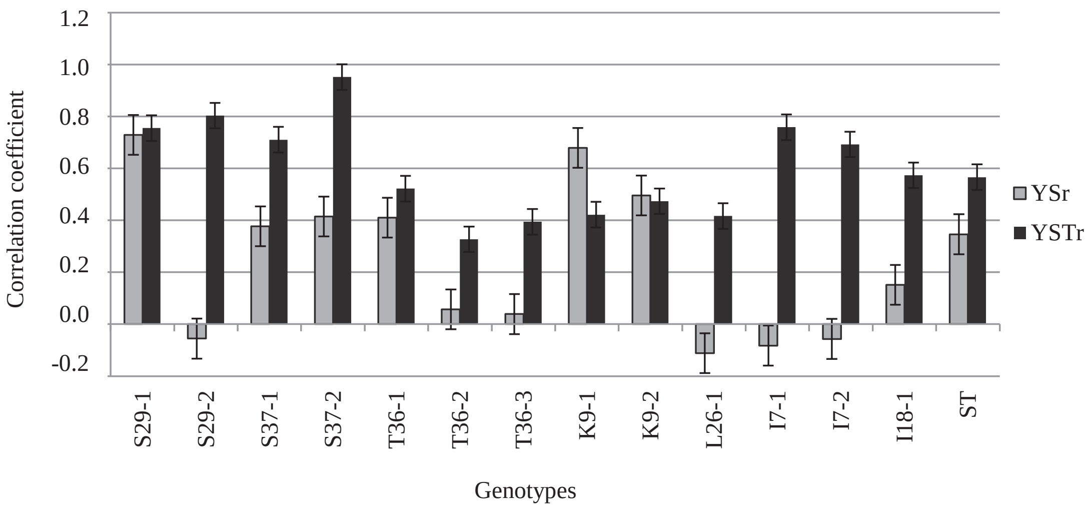

Figure 4. Correlation between yield of stem and sum of precipitation as well as between yield of seed and sum of precipitation for variety and lines of flax. (YS – yield of seeds; YST – yield of stem; r – correlation coefficient).

This year was characterized with a rapid drop of moisture from June to July (72.60 – 25.20 mm) and a rapid increase of moisture in August (124.80 mm) that created significant stress conditions for plants and stem development.

In the 1st and 2nd decade of May, a high (91% from long- term average) precipitation increase was observed and then a rapid drop in moisture (until 0 mm) that significantly affected plant emergence and development of flax stem occurred. In 2014, the line 'S29-2' (500 g m^{-2}) had the highest yield that was by 42% larger than the standard variety. According to the data, flax yield of stem could be majorly impacted by the amount of precipitation during the growing season and higher yield of stem results from lines of flax can be obtained in moist and cool weather. Berger (1969); Mankowski, Pudełko, & Kołodziej, (2013); Bakry *et al.*, (2014) also reported about similar results. Furthermore, by Nykter (2006) in order to obtain the best flax yield (fibre and seeds) the weather conditions should be stable from germination to the end of flowering. According to Bavec & Bavec, (2006), fibre flax belongs to hygrophytic plants with high transpiration coefficients between 400 and 780 as well as drought has a negative influence on plants from emergence to the flowering stage. In our case, genotypes had presented similar effect. Although results characterized the 2013 as the driest year, the two lines 'I18-1' and 'K9-1' exhibited higher yield of stem compared to 2014 and 2015 that had higher moisture content in vegetation period. These lines have the highest yield compared to other genotypes in moist as well as drought years. The higher average yield of stem has the line 'I18-1' (751.25 g m^{-2}) which is by 27% larger than the standard variety 'Vega 2'.

Comparing the data to a yield of stem, significantly higher yield of seeds was obtained in 2015 (by Fig. 3.). The year is characterized by high moisture content in May (about 38% higher than the long-time average) that has a positive impact on the smooth and good seed germination and plant development. In 2015, higher yield of seed has the line 'S29-2' (247.9 g m^{-2}) which is by 67% larger than the standard variety 'Vega 2'. Furthermore, the 2013 has the lowest yield of seed, which is characterized as the driest midst of the four investigational years. During this year, a significant negative impact on seed germination had drought in the 1st decade (0mm) of May. In 2013, higher yield of seed had the line 'L26-1' (114.2 g m^{-2}) which was by 50% larger than the standard variety 'Vega 2'. Comparing all years the most productive by average yield of seed was the flax line of 'S29-2' (165.85 g m^{-2}) which was by 42% larger than the standard variety 'Vega 2'.

Correlations are displayed between each flax variety and lines yield of seeds as well as stem and precipitations in Fig. 4. Positive relationships were found between the yield of stem and precipitation for all lines. In the analysis, significant positive relationships between the yield of stem and sum of precipitation of line 'S37-2', where r = 0.95 by significance of $p \leq 0.05$were identified were identified. The effects are related to the sum of precipitation level. Considering the relationships between the yield of seed and sum of precipitation - they are comparatively less sought by high moisture quantity, and presented analysis had a positive but no significant effect. Higher positive correlation for the yield of seeds has the line 'S29-1' (r = 0.73). Taking into account the productivity of yield rates and plasticity characteristics, the most

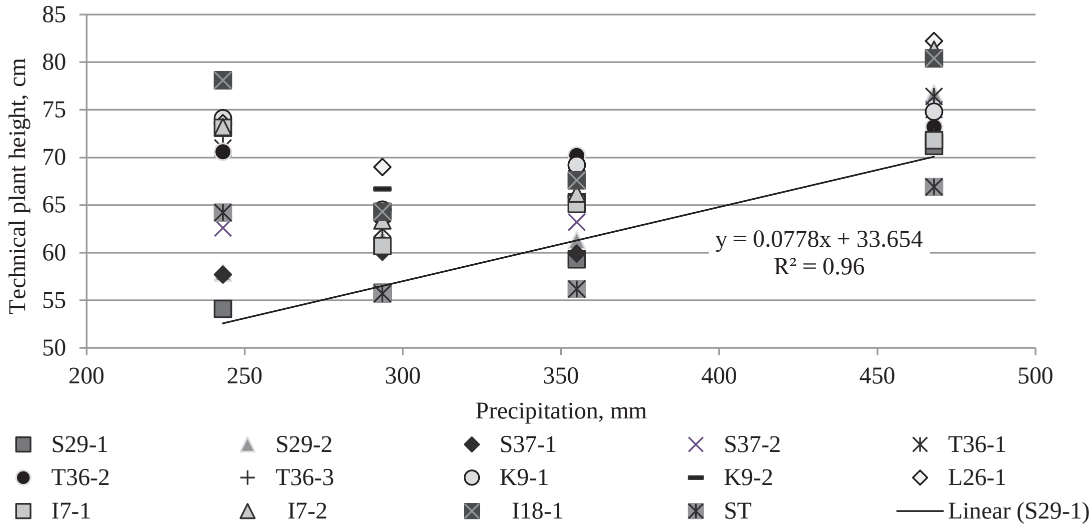

Figure 5. Relationships between technical plant height (cm) and sum of precipitation in four vegetation periods for each flax lines and variety. (2012 – 467.9 mm, 2013 – 243.1 mm, 2014 – 354.9 mm, 2015 – 293.5 mm).

valuable lines for fiber flax breeding were lines 'I18-1' and 'K9-1'. The line 'S29-2' that is more resistant to rapid changes precipitations during the growing season by the yield of stem and higher productivity of yield of seeds has good potential.

Results showed the regression line with positive relationship between the technical plant height and sum of precipitation during the growing season for all lines and variety (presented in Figure 5.). The results indicated that the technical plant of height increases against precipitation level. Results are confirmed by the results of Polonetskaya, Panifedova, & Sakovich, (2001). In case, statistical significant positive correlation has one line 'S29-1' $R^2 = 0.96$ and with $r = 0.98$ by significance of $p \leq 0.05$.

Evaluating the averages the highest technical plant height has the line 'L26-1' (73.05 cm) by 20% higher than the standard variety 'Vega 2'. Grauda, Stramkale, & Rashal (2004) proved that the most important trait for the fibre flax breeding is the technical plant height. This study presented similar results that the genotype with higher average yield of stem as the line 'I18-1' does not provide the highest technical plant length as the line 'L26-1'. In addition, the genotypes displayed different correlation in relation to sum of precipitation and statistical significance level presented for the yield of stem ('S37-2') as well as the technical plant height ('S29-1'). Result presents that yield formation was significantly influenced by the sum of precipitation and genotype.

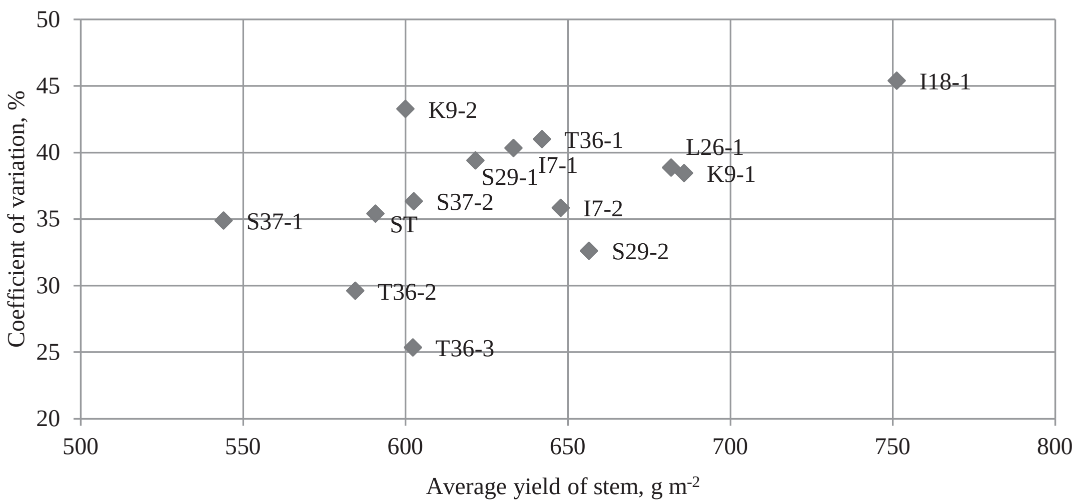

Figure 6. Coefficient of variation against average yield of stem for lines and variety of flax.

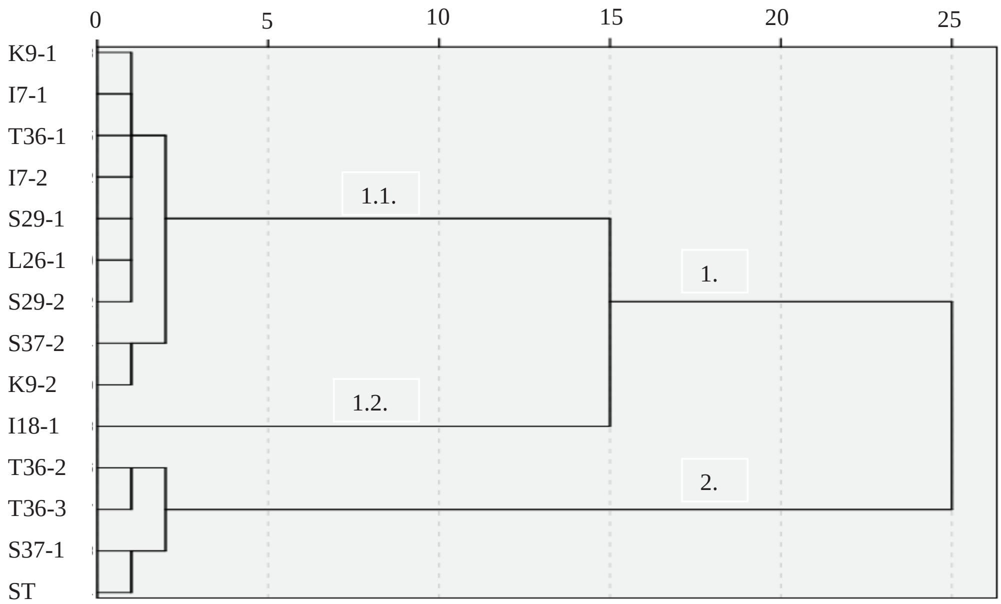

Figure 7. Dendrogram for 14 flax genotypes. (1 and 2 main group; 1.1 and 1.2 sub-group).

High yield stability usually refers to a genotype ability to perform consistently, whether at high or low yield levels by weather changes. In this case characterizing yield stability in a breeding program, an important criterion is heritability. According to Ortiz *et al*., (2001) coefficient of variation (CV) had the highest narrow-sense heritability (h^2 = 0.522) and analyzing grain yield results suggested that it may be possible to select simultaneously for high and stable yield by selecting out yielders that exhibit low CV. In our study coefficients of variation were not significantly correlated with average yields (by Fig. 6.). Some genotypes showed better stability than others. According to results, the higher yield of stem and higher CV has the line 'I18-1'from all genotypes. However, better potential has the lines 'L26-1', 'K9-1' or 'S29-1' that show high average yield of stem and more stable yield level to changing environmental conditions.

The cluster analysis of 14 genotypes was based on yield of stem and yield component (by Fig. 7.). According to the results, the genotypes were diverged in two main groups. The first main group was divided in two sub-groups. First sub-group of yield of stem and yield component in comparison to second main sub-group is more productive and contains 9 lines of flax ('K9-1', 'I7-1', '36-1', 'I7-2', 'S29-1', 'L26-1', 'S29-2', 'S37-2'and 'K9-2') . Second sub-group contains an independent line 'I18-1' that is characterized by the highest yield of stem and total plant height that is potentially different from other line after yield components and with largest distances 15. Dendrogram results indicated first main group with good potential for breeding program; consequently, it is a suitable group for producing fibre. Bakry *et al*., (2014) also found similar results: agronomic data of 49 flax varieties were evaluated, using cluster analysis that revealed the existence of variations among varieties. Therefore, cluster analysis is a suitable solution to group and select desirable genotypes for well-grounded selection as well for classifying the high yield genotypes with high component characters.

Conclusions

Overall, the higher yield of stem for all evaluated flax genotypes was obtained in the moisture and cool environmental conditions and suggesting that water stress was a major factor that influenced yield variation. Results showed that high-yielding cultivars can differ in yield stability, and suggested that yield stability and high yield of stem are not mutually exclusive. According to the results, it could be put forward flax line 'I18-1' (yield of stem 751.25 g m^{-2}) where higher yield of stem was obtained, but it was associated with low stability and specifically adapted to favorable environmental conditions. However, the line 'S29-2' (yield of stem 656.5 g m^{-2}) that showed higher yield productivity and resistance to stress factors (as rapid precipitation change) has higher stability. Most valuable by the average technical plant height was the line 'L26-1' (73.05 cm). Determination of correlation, regression, coefficient of variation and cluster analysis has provided effective selection criteria for creating new ecological plastic fiber flax varieties.

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