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Agronomic evaluation of regenerant oat (*Avena sativa* L.) lines

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Abstract

Mature embryo-derived somaclones regenerated from variety Kehlibar and breeding line 471-3 were analyzed for agronomic traits to evaluate the potential of tissue cultures to induce genetic variability in agronomic traits in oat (*Avena sativa* L.). The selected lines and their parents were evaluated in the experimental field of the Institute of Agriculture – Karnobat, Southeastern Bulgaria, during 2013/2014 and 2014/2015 growing seasons. Traits: plant height (cm), panicle length (cm), number of spikelets per panicle, number of grains per panicle, grain weight per panicle (g), 1000 grain weight (g), protein content, crude fat content and grain yield were studied. The results indicate significant differences among the regenerant lines and their parents in yield and yield related traits. Regenerant lines with lower plant height, longer panicle, higher number of spikelets and grains per panicle, higher grain yield and higher 1000 grain weight and higher protein and crude fat content compared to parents were founded.

Keywords: oat; *Avena sativa*; somaclonal variation; regenerant lines; agronomic traits

INTRODUCTION

Oat is listed among the major cereal crops in the world. It is ranked sixth after wheat, maize, rice, barley and sorghum. The oat grain is a major livestock feed. It is also used widely for human consumption, as a source of valuable nutrients. However, genetic variability of some agriculturally relevant traits is low, limiting the possibility of gains in oat grain yield and quality.

Biotechnological techniques can increase variability and help in the selection of genotypes useful in breeding programs. Certain genetic events seem to have an exceptionally high frequency *in vitro*, though this frequency may vary between species, genotypes, sources of explants and culture media (Larkin and Scowcroft, 1981). Some of the genetic events responsible for variation are genetic mutations, “transposon” activation, chromosome breakages, rearrangements, DNA modifications (Lee and Phillips, 1988; Evans, 1989; Skirvin et al., 1994; Karp, 1995; Bairu et al., 2011; Neelakandan and

Wang, 2012). Regardless of the mechanisms through which variation is induced, tissue culture may generate agronomic variation that is potentially useful in plant improvement.

There are reports that genetic and cytogenetic variation appears as a common characteristic in plants derived from tissue cultures of oat (Rines et al., 1986; Cummings et al., 1976; McCoy et al., 1982; Dahleen et al., 1991). Variations have been observed in different agronomic traits as flowering date, plant height, grain protein quantity, flag-leaf area, weight and number of seeds, and grain yield (Rines et al., 1986; Dahleen et al., 1991; Augustin et al., 2000).

Limited field studies have been performed to evaluate the extent of variability in agronomic traits exhibited in lines regenerated from tissue culture in oat. Although it was generally shown that a wide variation was found among the regenerant lines, very few lines were identified that significantly outperformed their parents in important agronomic characters. The objective of this study was to evalu-

ate agronomic traits in selected advanced regenerant oat (*Avena sativa* L.) lines.

MATERIAL AND METHODS

The regenerant lines R 11-1, R 11-2, R 11-3 and R 11-4 from variety Kehlibar and R 13-1 and R 13-2 from breeding line 471-3 were selected for evaluation of their field performance and grain quality characteristics in R6 and R7 generation. The regenerant lines are derived from tissue culture of mature embryos. Mature embryos after being excised from presoaked seeds were placed on MS medium containing 2 mg/L 2,4-D at 25°C under continuous illumination (6,000-8,000 lux). Calli were subcultured every 2 weeks in the same medium and maintained at 25°C under continuous illumination. When shoots were regenerated the calli with shoots were transferred to the MS hormone-free media for induction of adventitious roots. Regenerant lines that did not differ from the parents or with undesirable traits were discarded from R2 to R6 generation.

The selected lines and their parents were evaluated in the experimental field of the Institute of Agriculture – Karnobat, Southeastern Bulgaria. This research was conducted during 2013/2014 and 2014/2015 growing seasons. The experiments were organized in a Complete Block Design with 4 replications on plots of 10 m². Standard agronomic and plant protection practices were used.

Data were collected for plant height (cm), panicle length (cm), number of spikelets per panicle, number of grains per panicle, grain weight per panicle (g), 1000 grain weight (g). Protein content, crude fat content and grain yield per plot were recorded. The plot yield was converted to kg/ha.

The data were subjected to analysis of variance (ANOVA) and cluster analysis. ANOVA were obtained by SPSS 16.00 for Windows (SPSS Inc., 2007). The cluster analysis was performed using the program Statistica 7.0 (StatSoft Inc., 2004) that adopts Euclidean distance as a measure of dissimilarity and the Ward's method as the clustering algorithm (Ward, 1963) using the newly created variables after standardizing.

RESULTS AND DISCUSSION

The results of analysis of variance for grain yield and yield related traits in regenerant oat lines and their parents are given in Table 1. Highly significant differences were observed between genotypes and between environments and their interactions ($P < 0.01$) for grain yield and yield related traits. The genotype was the main factor controlling the number of spikelets per panicle, panicle length, 1000 grain weight and plant height, accounting for 90.24%, 89.80%, 73.32% and 70.48% of the total variance, respectively. Most of the total variance in number of grains per panicle, grain weight per panicle and

Table 1. Analysis of variance for grain yield and yield related traits in regenerant oat lines and their parents (2013/2014 - 2014/2015)

Traits	Sources of variation					
	Genotype		Year		Interaction	
	MS	SS, %	MS	SS, %	MS	SS, %
Plant height	655.28*	70.48	1251.39*	19.23	95.71*	10.29
Panicle length	81.38*	89.80	17.35*	2.73	6.77*	7.47
Number of spikelets per panicle	2559.52*	90.24	266.75*	1.35	238.63*	8.41
Number of grains per panicle	943.67*	40.65	3110.85*	19.15	933.15*	40.20
Grain weight per panicle	0.27*	22.43	3.39*	39.66	0.46*	37.91
Grain yield	13041126.56*	23.97	4525271.20*	58.24	1382330.13*	17.79
1000 grain weight	71.11*	73.32	0.23*	0.040	25.84*	26.64

MS - mean squares; SS - % of total sum of squares of genotype, year and their interaction; * $p \leq 0.1$;

grain yield was determined partially by the E, along with G, and GEI also showed influence. Analysis of variance suggested that grain yield, groat starch, and ash concentrations were more strongly affected by environment than by genotype. Doehlert et al. (2001) and Akcura et al. (2006) also founded that grain yield in oat was more strongly affected by environment than by genotype.

Mean values of grain yield and yield related traits in regenerant oat lines and their parents for the period 2013/2014 - 2014/2015 are presented in Table 2. Line R 11-1 had significantly taller plants, longer panicle with less spikelets and grains per panicle in comparison with parent variety Kehlibar. Regenerant line R 11-2 had shorter plants and panicle, less spikelets and grains per panicle and lower grain weight per panicle than a parent. Line R 11-3 had shorter plants, longer panicle, more spikelets per panicle and lower grain weight per panicle than Kehlibar. Line R 11-4 had taller plants, less spikelets and grains per panicle and lower grain weight per panicle as *compared* with the *parent variety*. All regenerant lines developed from Kehlibar showed a significantly lower grain yield than the parent variety. Line R 11-1 had significantly higher 1000 grain weight, whereas R 11-2 and R 11-3 showed lower 1000 grain weight than the parent.

Regenerant lines R 13-1 and R 13-2 were characterized with taller plants and longer panicle than parent line 471-3. Line R 13-1 showed more spike-

lets and grains per panicle, but lower 1000 grain weight and grain yield. The regenerant R 13-2 had higher 1000 grain weight and exceeded the parent line in grain yield by 12.5%.

The protein content in oat grain has been considered as an important trait for feed and food quality because of its nutritional significance. Studies have shown genotypic and environmental effects on oat protein content (Forsberg and Reeves, 1992; Doehlert et al., 2001; Peterson et al., 2005). The mean protein content of studied oat genotypes is presented in Figure 1. Higher protein content than parent Kehlibar (11.76%) was found in lines R 11-1 (12.59%), R 11-2 (12.34%) and R 11-4 (12.28%). Protein content of lines R 13-1 (11.27%) and R 13-2 (11.62%) exceeded that of the parent line (10.65%).

Oat contains much higher fat content than do other small grains (Youngs, 1986). Higher fat content is an advantage for animal feeding because of its higher caloric content. However, in food applications, higher fat concentrations are deleterious because of their potential for rancidity. Crude fat content in oat grains is genetically controlled, but also affected by the environmental conditions (Saastamoinen et al., 1989; Humphreys et al., 1994). In our study highest crude fat content was found in R 11-4 (7.16%), followed by R 13-2 (6.95%) (Figure 2). The lowest content of crude fat had R 13-1 (5.51%). Because of the negative correlation between protein and fat content (Forsberg et al., 1974; Doehlert et al., 2001),

Table 2. Mean values of grain yield and yield related traits in regenerant oat lines and their parents (2013/2014 - 2014/2015)

Genotype	Plant height, cm	Panicle length, cm	Number of spikelets per panicle	Number of grains per panicle	Grain weight per panicle, g	Grain yield, kg/ha	1000 grain weight, g
Kehlibar	87.63	16.15	33.28	73.31	2.30	6063	29.89
R 11-1	95.75	16.93	32.48	70.05	2.20	5373	31.49
R 11-2	83.25	15.00	29.95	64.46	1.82	5348	27.82
R 11-3	83.38	16.88	37.65	73.89	2.05	5744	28.10
R 11-4	90.00	16.20	31.71	63.72	1.84	5741	29.69
471-3	98.25	18.29	40.79	77.39	2.18	4414	29.35
R 13-1	106.13	20.52	84.43	98.56	2.16	3820	22.29
R 13-2	105.00	24.84	39.58	74.10	2.26	4965	31.77
<i>LSD 0.05%</i>	<i>4.31</i>	<i>0.36</i>	<i>0.42</i>	<i>0.88</i>	<i>0.21</i>	<i>416</i>	<i>0.35</i>

genotypes having simultaneously high protein and fat in grain are of *interest* in oat *breeding*. In our study R 11-2 and R 11-4 had relatively high protein and fat content in grain.

Based on grain yield, yield related traits and contents of protein and crude fat in grain, the dendrogram divided studied oat genotypes into three cluster groups (Figure 3). The first cluster included only one genotype R 13-1. This fact indicates that line R 13-1 was significantly different from the parent line and the remaining regenerant lines. The second cluster grouped the line R 13-1, parent line 471-3

and one line originated from variety Kehlibar – R 11-3. The third cluster consisted of the parent variety Kehlibar and lines derived of this variety – R 11-1, R 11-2 and R 11-4. This grouping indicate that regenerant lines exhibited dissimilarity in studied traits not only from the parent, but also among themselves.

The results of our study indicate significant differences among the regenerant lines and their parents in grain yield and yield related traits. Regenerant lines, superior to parents were found for all traits except grain weight per panicle. Dahleen et al.

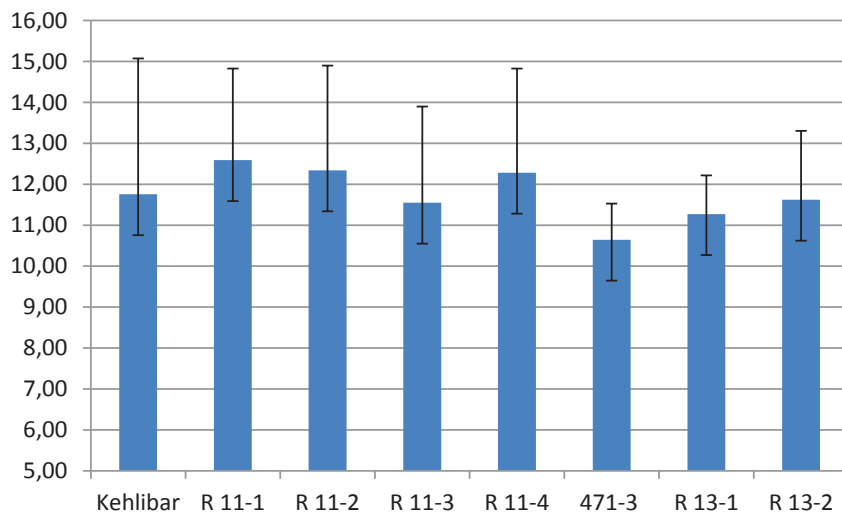


Figure 1. Mean values (\pm standard deviation) of protein content of oat genotypes (2013/2014 - 2014/2015)

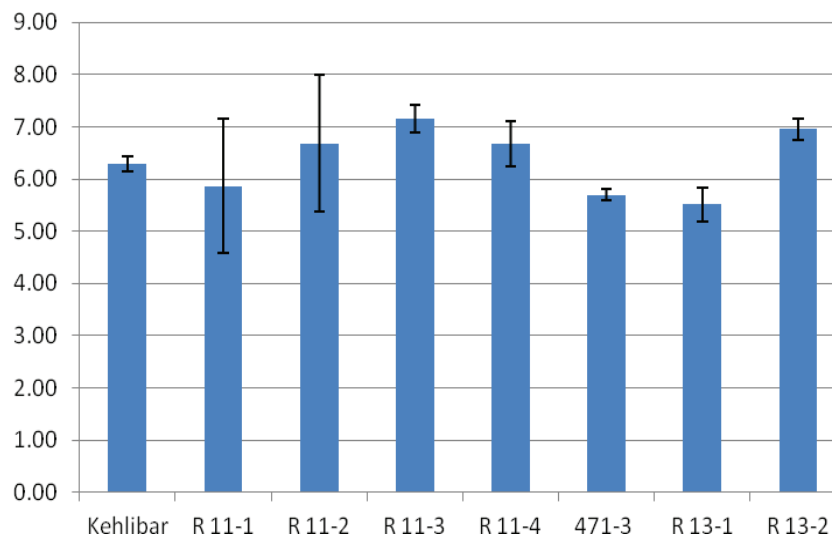


Figure 2. Mean values (\pm standard deviation) of crude fat in grain of oat genotypes (2013/2014 - 2014/2015)

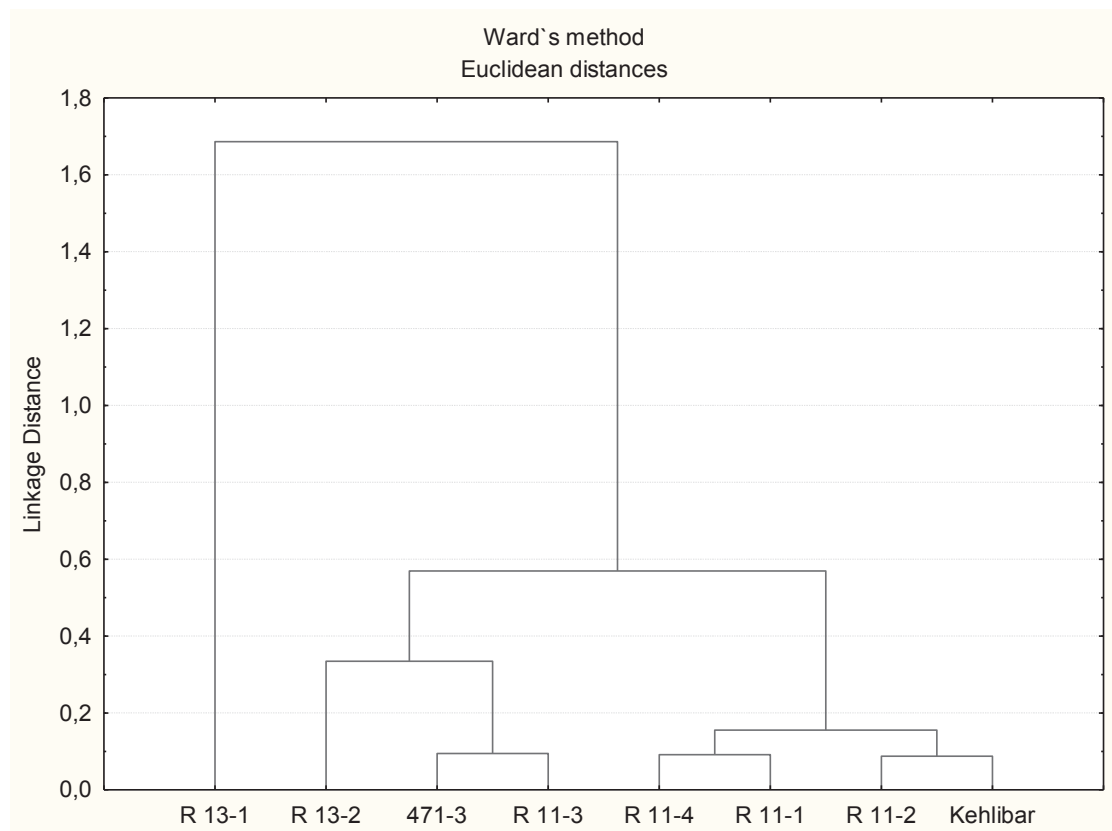


Figure 3. Dendrogram of 8 oat genotypes based on grain yield, yield related traits and contents of protein and crude fat in grain

(1991) have studied agronomic trait variation in oat lines derived from tissue culture and they reported that although agronomically less desirable changes were more frequent than desirable changes, lines with increases and lines with decreases were found for each trait.

CONCLUSIONS

Significant differences among the regenerant lines derived from *mature embryo* explants and their parents in grain yield and yield related traits have been identified. Regenerant lines with lower plant height (R 11-2 and R11-3), longer panicle (R 11-1, R 11-3, R 13-1 and R 13-3), higher number of spikelets per panicle (R 11-3 and R 13-1), higher number of grains per panicle (R 13-1), higher grain yield (R 13-2) and higher 1000 grain weight (R 11-1 and R 13-2) compared to parents have been observed. Lines derived from tissue culture with higher protein and fat content in grain were also found.

The results of the present study showed that somatological variation in oat as an additional source of genetic diversity of agronomically important traits can be used as an integral part of a continuous breeding program.

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