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APPLICATION OF AUGMENTED DESIGNS FOR FIELD EVALUATION OF BREAD WHEAT DOUBLED HAPLOID LINES: A PRELIMINARY REPORT

Demetrios DIKEOPHYLAX[1], Iordanis OSSOUTZOGLOU[1], Chryssanthi PANKOU[1], Athanasios G. MAVROMATIS[1], Anastasios S. LITHOURGIDIS[1], Ioannis N. XYNIAS[2*]

[1]Laboratory of Genetics and Plant Breeding, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
[2]School of Agricultural Technol. & Food Technol. and Nutrition, Western Macedonia University of Applied Sciences TermaKontopoulou, 53 100 Florina, Greece
*Corresponding author:ixynias@teiwm.gr; ioannis_xynias@hotmail.com

ABSTRACT

Doubled-haploid is an effective method to produce 100% homozygous lines in a single generation accelerating the release of new varieties and reducing the corresponding expenses. However, the existing problem in cases where the adequate quantity of seeds is limited is the inability to evaluate new germplasm in replicated experiments. In his attempt to confront this problem, Petersen proposed in 1985 the evaluation of new germplasm to be based on its division in blocks and selection to be performed regarding the yield of the randomly repeated control in each block. The aim of the present study was to use the aforementioned method to evaluate preliminary 37 doubled-haploid lines (DHL). The parental varieties of the DHLs,Greek cultivars “Acheloos” and “Vergina”, were used as controls. For the purpose of the study, 35 main spikes, one form each DHL and control, were used. The length of the spikes was measured, the number of spikelets was counted, and the 1000 kernel weight and total yield were recorded. The data analysis revealed that only one line exceeded the mean number of spikelets of the controls, one exceeded the mean yield of the controls and two exceeded the mean 1000 kernel weight of the controls (one was even better than the best control). The reported results indicate the presence of valuable genetic variability among the DHL after crossing cultivars “Acheloos” x “Vergina”. Further research is needed, after DHLs multiplication, using more plants and locations to draw more reliable conclusions.

Keywords: *block, control cultivar, traits, analysis, genetic variability.*

INTRODUCTION

The main problem in evaluating segregating generations of new germplasm is the inadequate quantity of seeds to conduct replicated experiments (Fehr, 1987). For this to be faced, various approaches have been proposed. Papadakis (1935)

recommended the use of the adjusting control in order to select the best genotypes. Fasoulas suggested the honeycomb method to overcome this problem (Fasoulas, 1973). This method uses small quantities of seeds, which must be sownat least one meter apart to avoid competition of the resulted plants and has replaced plot evaluation with single plant evaluation. Another interesting designto evaluate large numbers of new selections was proposed by Petersen (1985). Petersen revised the "Augmented designs" that were originally developed by Federer (1961) and Federer and Ragavarao (1975).According to the aforementioned designs,the evaluationcan be based on the division of the new germplasminto blocks and selection to be performed regarding the yield of the randomly repeated control or controls within each block (Petersen, 1985). This will enable the breeder to perform a valid statistical analysis despite the large number of new selections.

Anther culture is a well-known and effective method to produce 100% homozygous lines in a single generation accelerating the release of new varieties and reducing the corresponding expenses (Henry and de Buyser, 1990;Deyao and Xigan, 1990; Hussain *et al.*, 2012). However, as in the case of segregating generations, the main problem isstill the inadequate quantity of seeds limiting the inability to evaluate the produced doubled haploid lines in replicated experiments. Furthermore, over locations evaluation seems to be impossible resulting in a considerable delay of estimating the yield (mainly) and quality potential of the selected material. In this case, the Petersen's approach is also suitable since the parental lines can be used as the repeated controls.

The aim of the present study was to use the augmented designs to evaluate preliminary 37 doubled-haploid lines (DHL) produced after anther-culture of the F_1 generation after crossing the Hellenic commercial cultivars "Acheloos" x "Vergina".

MATERIALS AND METHODS

A. Plant material

For the purpose of the study 37 doubled haploid (DH) lines produced by anther-culture of the F_1 generation after crossing the Hellenic commercial cultivars "Acheloos" and "Vergina" were used. The parental cultivars of the F_1 generation were originally used in the cross because "Acheloos" is a high yielding potential cultivar, responding well to anther-culture and cultivar "Vergina" is a broad adapted variety, but with null-response to anther-culture (Zamani*et al.*, 1998). The 37 DH lines were described in the past by Rigas*at al*., (2008) but without and statistical evaluation due to small quantities of seeds. The DH lines and the control cultivars were sown in autumn 2015 in a field at the University Farm of Thessaloniki, Northern Greece, in a loam (L) soil (TypicXerorthent) with pH 7.8 organic matter content 13.4 g kg^{-1},N-NO_3 38 mg kg^{-1},P (Olsen) 26 mg kg^{-1} and K 156.6 mg kg^{-1} (0 to 30 cm depth). Seedbed preparation included mouldboard plough, disc harrow and cultivator. Nitrogen and P_20_5 at 80 and 40 kg ha^{-1}, respectively, were incorporated into the soil as diammonium phosphate (20-10-0) before sowing. The crop was kept free of weeds by hand hoeing when necessary.

B. Method

Despite the considerable effort to keep the plants in good farming conditions, a severe attack by birds resulted in unequal number of plants per DH line. For this it was decided 35 main spiked from each DH line to be used in the study. The following traits were evaluated: spike length, number of spikelets per spike, yield in g and 1000 kernel weight in g. The DH lines were divided in four blocks and the parental cultivars "Acheloos" and "Vergina" were used as controls. In Augmented designs the effect of each block is estimated by the formula:

$r_j = 1/c(B_j\text{-}M)$,

where c: is the number of controls, B_j: is the sum of all controls in j block and

M: is the sum of all means.

The 5% generalLSD for comparing an adjusted selection yield with the mean yield of a controlis calculated by the formula

$$LSD = t_{0.5,3}\sqrt{Svc^2}$$

df= is the block number-1.

Thedifferencebetween an adjusted selection yield and a control mean is

$Svc^2 = (b\text{-}1)(c\text{-}1)/bc$,

where b: is the number of blocksand c: is the number of controls.

The LSD value of each block is estimated by adding the value r_j of each block to the general LSD value. In order a DH line to be selected it has to exceed the value of LSD + the mean value of the controls (*) or the value of LSD + the mean value of the best control (**)

Table 1.Spike length, number of spikelets per spike, yield in g and 1000 kernel weight of the 37 DH lines and the controls.

S. No.	Genotype	Number of spikelets	Spike length	Yield (g)	1000 kernel weight
A1	Acheloos	18.37	10.39	61.9	37.85
2	Vergina	19.28	12.28	32.7	24.65
3	24	19.7	13.09	27.6	19.95
4	25	17.7	8.6	31.6	33.83
5	26	18.9	8.04	38.0	38.0*
6	27	20.2	8.62	40.5	41.85**
7	52	18.6	11.4	49.9	38.28*
8	54	22.5	10.12	76.0*	32.91
9	56	23.4	9.92	58.7	25.42
10	57	21.9	10.1	40.9	29.13
11	59	22.3	9.62	44.9	30.58
B12	Acheloos	20.1	10.12	46.6	36.57
13	Vergina	14.84	11.54	31.0	33.01
14	60	23.2*	10.02	53.7	29.28
15	90	22.1	9.9	54.3	29.61
16	106	20.9	11.78	50.7	33.01
17	107	21.9	10.3	44.9	28.4
18	108	21.7	10.7	33.0	24.3
19	120	21.2	10.9	47.3	34.75
20	121	22.5	12.91	43.9	24.33
21	122	22.4	12.5	41.6	26.93
22	123	22.14	12.6	47.6	28.79
C23	Acheloos	19.26	9.48	45.9	35.57
24	Vergina	22.1	12.35	45.9	29.45
25	125	22.4	12.4	45.2	24.88
26	126	18.51	11.97	55.5	36.29
27	127	23.03	13.2	36.1	24.68
28	128	17.9	11.37	24.3	21.83
29	151	16.9	10.9	34.3	30.94
30	152	19.2	9.45	48.8	31.02
31	156	17.85	10.7	36.0	30.72
32	157	19.2	10.8	44.7	32.43
33	173	15.0	10.0	26.7	26.98
D34	Acheloos	19.25	15.25	57.4	34.48
35	Vergina	16.11	11.4	22.8	24.91
36	191	17.4	9.7	36.2	26.2
37	255	15.54	11.48	16.0	24.11
38	262	18.4	11.7	36.7	34.4
39	282	19.46	10.85	21.6	24.8
40	295	20.8	10.88	36.9	27.33
41	297	18.54	11.35	30.9	25.85
42	306	19.37	10.55	32.0	35.21
43	308	18.54	10.58	29.6	29.29
44	314	19.31	11.08	22.8	31.12
45	352	17.57	10.85	29.9	25.13

* DH line differing from the mean value of the controls,

** DH line differing from the best control.

Table 2. LSD value for each examined trait according to mean of controls and best control.

Block number	LSD + mean of controls				LSD + best control			
	No of spikelets	Spike length cm	Yield in g	1000 kernel weight g	No of spikelets	Spike length cm	Yield in g	1000 kernel weight g
A	23.92	15.45	68.61	37.03	24.5	15.75	78.53	41.08
B	22.56	14.94	60.11	40.58	23.15	15.24	70.03	44.63
C	25.77	15.03	67.13	38.30	26.36	15.33	77.05	42.35
D	22.77	17.44	61.33	35.70	23.36	17.74	71.25	39.75

RESULTS AND DISCUSSION

It is well established that crossing two cultivars, one responding well and one non-responding, results in a F_1 generation which responds well to anther-culture (Zamani*et al*., 2000). This permits to exploiting the valuable traits of the non-responding cultivar. Thus, it was possible to benefit the good response of cultivar Acheloos and produce well responding DHLs after the aforementioned cultivar to the non-responding cultivar Vergina. Indeed F_1 plants were produced after crossing the aforementioned cultivars but the quantity of seeds produced was not sufficient to conduct replicated experiments. For this, application of an approach like augmented designs as inevitable.

The analysis of the obtained data revealed that in number of spikelets per spike despite the recorded variability, only one DH line (No 60) exceeded the mean value of the controls (Table 1). The LSD values per each examine trait are presented in Table 2. The values ranged from 15 spikelets per spike in DHL No 173 to 23.2 in DHL No. 60. In spike length, the values ranged from 8.6 cm in DHL No. 25 to 13.09 cm in DHL No. 24. Again, despite this variability the recorded differences were not also significant. In yield, the lowest value was recorded in DHL No. 255 (16g) and the highest in DHL No. 54 (76g). This last line differed significantly from the mean yield of the controls. Finally, regarding the 1000 kernel weight the values ranged from 19.95g in DHL No. 24 to 41,85b in DHL No. 27. In this trait, two DHLs were significantly better than the mean value of the controls and one line (No. 27) was significantly better than the best control. Dramalis *et al.* (2006) also reported the presence of valuable variability in the offspring of DH lines. In this last study, honeycomb selection was used.

Example of estimating LSD value per examined trait: the case of yield

Control	Block number				Total yield	Mean yield
	A	B	C	D		
Acheloos	61.9	46.6	45.9	57.4	211.8	52.95
Vergina	32.7	31.0	45.9	22.8	132.4	33.1
TOTAL	94.6	77.6	91.8	80.2	344.2	86.05
				Control	mean	43.03

Block effect: $r_j = 1/c\ (B_j - M)$, where B_j: sum of all means in j block and M: sum of all means

$r_1 = (96.4\text{-}86.05)/2 = 4.275$

$r_2 = (77.6\text{-}86.05)/2 = -4.225$

$r_3 = (91.8\text{-}86.05)/2 = 2.8$

$r_4 = (80.2\text{-}86.05)/2 = -3$

Analysis of variance table

Source	DF	Sum of squares	Mean squares	F-value	Prob.
Varieties	1	788.05	788.045	6.59	0.0826
Replications	3	105.90	35.298	0.30	0.8284
Error	3	358.54	119.512		
Total	7				

$Svc^2 = (b\text{-}1)\ (c\text{-}1)\ MSE/bc = (4\text{-}1)(2\text{-}1)119.512/8 = 44.82$

$LSD = t_{0.5,\ 3}\sqrt{Svc^2} = 3.182\sqrt{44.82} = 21.30$

LSD 1st block =21.3 + 4.275 = 25.575

LSD 2nd block =21.3- 4.225 = 17.075

LSD 3rd block = 21.3+2.8 = 24.1

LSD 4th block = 21.3 – 3 = 18.3

LSD + mean of controls	LSD + best control
Block A = 25.58 + 43.03 = 68.61	Block A = 25.58 + 52.95 = 78.53
Block B = 17.075 + 43.05 = 60.11	Block B = 17.075 + 52.95 = 70.03
Block C = 24.1 + 43.03 = 67.13	Block C = 24.1 + 52.95 = 77.05
Block D = 18.3 + 43.03 = 61.33	Block D = 18.3 + 52.95 = 71.25

CONCLUSIONS

The results presented in the study support the existence of genetic variability between the doubled-haploid lines produced after anther-culture of the cross “Acheloos” x “Vergina”. The parental cultivars were used as controls and this was the main difference from the classic augmented design analysis. Application of the augmented designs was found suitable enough in distinguishing genotypes in cases where the number of seeds excludes the establishment of replicated experiments. However, the described procedure must be repeated after multiplying the doubled-haploid lines involving more plants in the experiment. This will ensure greater credibility in drawing conclusions and in selecting the most promising line.

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