EVALUATION OF POLLINATORS (*ZEA MAYS* L. AND *HORDEUM BULBOSUM* L.) FOR WHEAT AND BARLEY HAPLOID PRODUCTION

Alexandrina Mihăilescu and Aurel Giura[1)]

ABSTRACT

Main genetic and environmental factors affect haploid induction by biotechnological systems, based on genome elimination in intergeneric (*Triticum aestivum* L. x *Zea mays* L) and interspecific (*Hordeum vulgare* L. x *Hordeum bulbosum* L.) crosses in wheat and barley. Among genetic factors the specific genotypic reaction of female form and pollen source in different *in vivo* and *in vitro* stages could be considered essential. This paper presents genotype influence of *Z. mays* and *H. bulbosum* on the crossing ability with *T. aestivum* and respectively *H. vulgare* genotypes and the genomic interactions in these crosses. Five pollen sources of *Zea mays* (three hybrids and two pollen mixtures) were tested in greenhouse and five pollinators of *H. bulbosum* diploid (two clones, two crossing reciprocal selections and one pollen mixture) in both greenhouse and field conditions, in 1996. The crossing rates were estimated by the following parameters: S/S (seed setting), SD/F (dissected seeds/100 pollinated florets), SD/S (dissected seeds/100 formed seeds), E/F (developed embryos/100 pollinated florets), E/SD (developed embryos/100 dissected seeds), ED/E (differentiated embryos/100 developed embryos) and HPE (haploid production efficiency, respectively P/F, regenerated haploid plants/100 pollinated florets). The results provided conclusive evidence for genotype significant differences among *Z. mays* and *H. bulbosum* pollinators regarding the crossability with wheat and barley. In wheat x *Z. mays* crosses, Bicaz hybrid was the most efficient for S/S and SD/S and Bicaz and Fundulea 102 hybrids and the pollen mixtures for E/F. The most substantial contribution of pollen sources in barley x *H. bulbosum* crosses was noticed with the diploid clone Cb 2920/4 and two crossing reciprocal selections from (Cb 2929/1 x Cb 2920/4) and (Cb 2920/4 x Cb 2929/1) hybridizations. These preliminary results will be useful in future selection of *Zea mays* and *Hordeum bulbosum* genotypes with high haploid induction ability in crosses with wheat and barley.

Key words: genotype influence, genome interactions, haploid production efficiency, *Hordeum vulgare*, *Hordeum bulbosum*, *Triticum aestivum*.

INTRODUCTION

The crossability in intergeneric *Triticum aestivum* L. x *Zea mays* L. and interspecific *Hordeum vulgare* L. x *Hordeum bulbosum* L. crosses and haploid induction in wheat and barley are markedly influenced by many genetic and environmental factors.

The most important genetic factors are represented by: genic control of crossability between *T. aestivum* and *Z. mays* and *H. vulgare* x *H. bulbosum* by homeologous loci *Kr* and *Inc* (localized on wheat 5A and 5B chromosomes and on barley homeologous 5H (Pickering and Devaux, 1992; Pedersen et al., 1996; Wang et al., 1996); specific genotypic reactions of mother forms and pollen sources in different *in vivo* stages (Pickering, 1983; Pickering and Rennie, 1990; Mihăilescu et al., 1995); genetic control of the response to the maximum stress environment represented by *in vitro* embryo culture (Mihăilescu et al., 1996).

The most important environmental factors involved in crossability seems to be: temperature during fertilization and paternal genome elimination (Pickering, 1984; Mihăilescu et al., 1996); temperature during *in vivo* applying of plant growth regulators (PGR) (Devaux, 1986; Mihăilescu et al., 1994); interaction of exogen PGR with wheat and barley specific endogen PGR contents (Ganeva et al., 1995).

In crosses between *H. vulgare* (genome V) and *H. bulbosum* (genome B), the compatibility depends on genomic ratio and genic interactions between the two species.

First of all, 1V:1B genomic ratio is the most favorable for *bulbosum* genome elimination and *vulgare* haploid embryo formation (Lange, 1971). On the other hand, the dominant gene *Inc* (on 5H chromosome) seems to control the crossability between the two species and some other major genes on 2H and 3H chromosome regulate selective elimination of *bulbosum* genome. There are several neutralizing genes in *bulbosum* genome which could counteract the elimination process (Pickering, 1983; Thomas and Pickering, 1985).

Crossability between wheat and maize is not so much affected by the *Kr* allelic system, which strongly controls the cross compatibility of wheat with alien species as *Secale, Elymus, Haynaldia, Aegilops, Hordeum*, etc. (Laurie and Bennett 1987; Simpson et al., 1980; Sitch and Snape, 1986). However, it seems that

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there are some genotypic differences of the maize pollen sources (Giura, 1994, 1995; Suenaga, 1996).

The purpose of the study was the evaluation of the pollen sources of *Zea mays* L. and *Hordeum bulbosum* L. for haploid induction in wheat and barley and selection of the most efficient pollinators.

MATERIALS AND METHODS

A number of 16 barley genotypes, lines and varieties (3 winter six-rowed, 8 winter two-rowed and 5 spring two-rowed) were included in this study carried out in 1996 in both greenhouse and field conditions.

Five pollen sources of *H. bulbosum* diploid (2n=2x=14)*: two clones PBIR 33 – (Hb1) and Cb 2920/4 – (Hb2), two crossing reciprocal derivates (CRD) from two combinations Cb 2929/1 x Cb 2920/4 – (Hb3) and Cb 2920/4 x Cb 2929/1 – (Hb4) and one pollen mixture (Hb5) were tested in crosses with *H. vulgare*.

A set of 15 wheat genotypes (12 F_1 hybrids and 3 varieties) were crossed in greenhouse with five maize pollinators represented by: two pollen mixtures of unknown origin (1 and 2) and three hybrids: Fundulea 102 (3), Suceava 97 (4) and Bicaz (5).

The detailed protocols for the main stages, modified at RICIC - Fundulea in wheat x maize and barley x *bulbosum* systems were described in previous papers by Giura (1994,1995) and Mihăilescu et al. (1993, 1994).

The crossing capacity and embryo formation were evaluated by the following parameters: S/S (seed set), SD/F (seeds dissected per 100 pollinated florets or seeds of quality), SD/S (seeds dissected per 100 formed seeds), E/F (developed embryos per 100 pollinated florets), E/SD (developed embryos per 100 dissected seeds) and ED/E (differentiated embryos per 100 developed embryos).

The embryo germination and plant regeneration were estimated by: EC/E (cultivated embryo per 100 developed embryos), EG/EC (germinated embryos per 100 cultivated embryos) and HPE (haploid production efficiency = SD/F x EC/SD x P/EC, after Hayes and Chen, 1989, where P means regenerated plants).

At least 10 spikes from each wheat and barley combination were analysed.

Analysis of variance was used to evaluate the influence of pollen sources on haploid production parameters in wheat and barley.

RESULTS AND DISCUSSIONS

The amount of biological material manipulated in barley x *H. bulbosum* crosses was represented by: 1037 processed spikes, 15884 pollinated florets (F), 7,060 formed seeds (S), 5,691 seeds of quality (seeds submitted to dissection, SD), 2,403 developed embryos (E), 2,252 cultivated embryos (EC), from which 1,694 were differentiated embryos (ED), 1,372 germinated embryos (EG) and 565 regenerated green haploid plants (P).

Similarly, in wheat x maize a number of 1,122 spikes, 26,751 pollinated florets, 22,466 formed seeds, 16,429 seeds of quality, 4,305 developed embryos, 2,920 cultivated embryos, 2,220 germinated embryos were processed and 1,821 green haploid plants were regenerated.

Results concerning the influence of *bulbosum* pollinators on haploid induction component parameters in barley are presented in Table 1.

In winter six-rowed barley, Hb2 and Hb3 pollinators gave generally superior values for all parameters of crossability and embryo formation. In winter two-rowed barley the most efficient pollinators seemed to be Hb2, Hb3 and Hb4. In spring two-rowed barley, better values for some of the parameters were registered for Hb1, Hb4 and Hb5 pollen sources.

F values for crossing capacity and embryo formation parameters in *H. vulgare* x *H. bulbosum* indicate that the pollinators influenced significantly two of the most important

* * The two hybrid selections and one clone, Cb 2920/4 of *H. bulbosum* were kindly provided by Dr. R. Pickering - New Zeeland

Table 1. Influence of *bulbosum* pollinators on haploid induction parameters

Pollinator	Crossability and embryo formation					
	S/S	SD/F	SD/S	E/F	E/SD	ED/E
Winter six-rowed barley						
Hb1	37.6	30.4	77.3	9.5	32.1	55.9
Hb2	42.0	36.2	81.5	14.0	34.9	52.8
Hb3	40.1	33.0	84.5	13.8	35.4	49.0
Hb4	39.6	29.0	69.5	10.4	31.0	43.8
Hb5	36.0	15.4	53.4	3.4	18.3	28.6
Winter two-rowed barley						
Hb1	42.2	32.8	71.0	15.9	47.4	44.1
Hb2	42.9	37.9	84.4	17.7	49.7	60.0
Hb3	49.5	40.5	78.8	19.4	47.8	58.5
Hb4	39.5	33.5	84.5	18.4	58.5	63.7
Hb5	47.2	41.9	87.0	15.8	36.8	55.8
Spring two-rowed barley						
Hb1	44.2	39.0	83.4	17.7	36.9	40.7
Hb2	-	-	-	-	-	-
Hb3	44.0	34.7	74.7	13.9	36.6	47.0
Hb4	42.1	35.7	80.9	15.3	45.9	59.7
Hb5	49.2	43.6	85.3	7.7	13.4	32.5

* Data from more extensive experiment (41 *vulgare* genotypes)

Table 2. F values for crossability and embryo formation parameters in *H.vulgare* x *H.bulbosum*

Sources of variation	S/S	SD/F	SD/S	E/F	E/SD	ED/E
Winter six-rowed barley						
Maternal genotype (G)	2.74	3.24*	2.44	3.29*	4.66*	1.50
Pollinator (P)	0.23	2.38	3.71**	2.54*	1.02	1.04
G x P	1.20	2.03*	1.44	1.32	1.00	1.67
Winter two-rowed barley						
Maternal genotype (G)	0.53	0.67	0.39	6.00***	5.50***	2.53*
Pollinator (P)	0.59	0.84	0.20	0.81	1.36	1.69
G x P	0.31	0.56	0.20	0.79	0.56	1.43
Spring two-rowed barley						
Maternal genotype (G)	0.94	0.80	0.78	2.82	1.25	4.76
Pollinator (P)	0.27	0.57	0.92	1.87	5.05	1.99
G x P	0.61	0.76	1.19	0.78	1.56	2.79

*, **,*** - Significant for $P< 0.05$, 0.01 and 0.001, respectively

parameters, SD/S and E/F only in winter six-rowed barley (Table 2).

On the other hand, maternal genotypes influenced significantly some of crossability parameters (SD/F, E/F, E/SD and ED/E) in both winter six- and two-rowed barley.

Spring two-rowed barley was by far the most crossable type, no significant effects of both pollinators and maternal genotypes on analysed parameters being registered.

Pickering (1983, 1984) developed the most exhaustive and accurate researches concerning the influence of parental phenotypes, *H. vulgare* and respectively *H. bulbosum*, on efficiency rates in all *in vivo* and *in vitro* stages of haploid induction system.

Our results were largely confirmed by these and other studies and have clearly indicated that both parents markedly affected seed setting and rates of embryo formation and differentiation, whereas seed quality was more influenced by female parent (Mihăilescu et al., 1995). Following embryoculture the influence of female partner was more evident as compared with pollen source which could be indirectly assumed (Jensen, 1983; Mihăilescu et al., 1996)

Haploid production efficiency (HPE) is the most synthetic parameter for estimation the rate of haploid induction in barley. Results showed that the pollinators influenced HPE due to their both direct (crossability) and indirect (*in vitro*) effects (Figure 1).

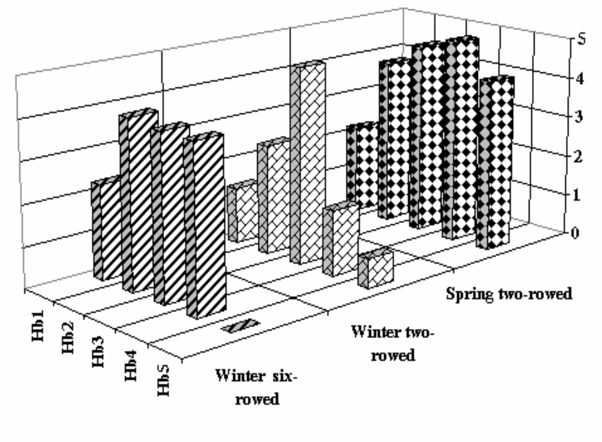

Figure 1. Influence of pollen sources on haploid production efficiency (HPE) in barley (mean of 41 genotypes)

According to the HPE values, ranking of pollinators was generally in concordance with that found for crossability (Table 3). After HPE mean values the best pollinators in crosses with winter six-rowed barley were Hb2, Hb3 and Hb4. In winter two-rowed barley the highest HPE values were given by Hb2 and Hb3. The pollinators Hb2, Hb3, Hb4 and Hb5 in crosses with spring two-rowed barley determined better haploid induction rates.

Table 3. HPE ranking of pollinators

Barley form	Crossability	HPE
Winter six-rowed	Hb2, Hb3	Hb2, Hb3, Hb4
Winter two-rowed	Hb2, Hb3, Hb4	Hb2, Hb3
Spring two-rowed	Hb1, Hb4, Hb5	Hb2, Hb3, Hb4, Hb5

Three of the five pollen sources, the clone Cb 2920/4 (Hb 2) and CDR Cb 2929/1 x Cb 2920/4 (Hb 3) and Cb 2920/4 x Cb 2929/1 (Hb4), gave generally in our experiment superior values for all crossability and embryo formation parameters and for HPE in both winter and spring barley, two- and six-rowed forms.

These sources, selected and intensively tested by Pickering (1983, 1984), Pickering and Rennie (1990) and Pickering and Devaux (1992), were also cited in other reports, as giving the best results, mainly in crosses with spring two-rowed barley varieties and are now largely utilized by private companies in doubled haploid production programme.

Regarding the use of pollen from individual clones or collected from many sources (mixtures), the present report is contradictory. Our results showed that pollen mixture was a poor haploid induction source, especially in winter six and two-rowed barley. A possible explanation of this situation could be the preferential participation of one of the mixture component in fertilization. Pickering (1984) and Pickering and Devaux (1992) recommended the use of individual pollination, with the two best *H. bulbosum* sources available, on opposite sides of the same spike.

In wheat mean values of parameters estimating *in vivo* influence of pollinators showed notable differences among maize sources (Table 4).

Table 4. In vivo influence of maize pollinators on haploid induction parameters

Pollinator (mixture)	S/S	SD/F	SD/S	E/F	E/SD	ED/E
A1	77.2	54.6	70.8	17.9	32.7	84.0
A2	82.8	59.1	71.3	16.7	28.2	92.9
Fundulea 102	85.4	65.0	76.1	17.5	27.0	89.3
Suceava 97	78.4	53.4	68.1	15.1	28.3	95.3
Bicaz	93.9	69.6	74.1	6.6	9.4	100

According to analysis of variances, significant differences among pollen sources of *Z. mays* were detected; the pollen mixture 1 produced significantly lower S/S and SD/F as compared to the other sources (Figure 2).

For these two parameters, the best results (significantly higher) were obtained with source 5 (hybrid Bicaz).

Pollen sources 1,2,3 and 5 gave similar results for E/F parameter, while source 4 produced significantly lower value.

Although, many results revealed that the incompatibility genes *Kr1* and *Kr2* were not strictly involved in wheat x maize crosses than in other intergeneric hybridizations, there were however some manifested differences among maize pollinators (Laurie and Bennett, 1987; Suenaga, 1996; Giura, 1994, 1995). Therefore, it seems that the selection of maize varieties, inbreds or hybrids (from the material developed and adapted in a given region), showing high ability for haploid induction in crosses with wheat and shedding large amount of pollen, might be possible.

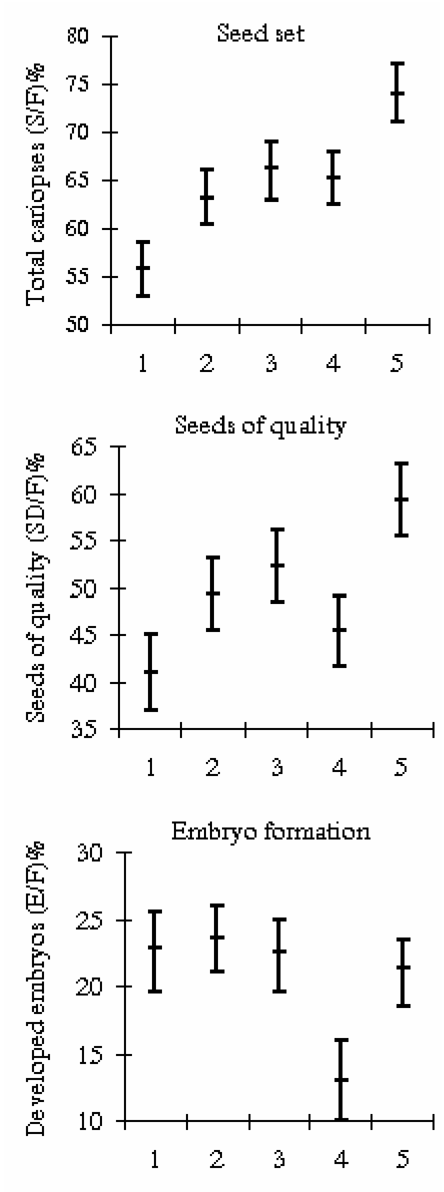

Figure 2. Influence of pollen sources (1,2=mixture; 3=F 102; 4=Sv.97; 5=Bicaz) on some efficiency parameters in wheat x maize haploid production

The influence of both parents on the efficiency of DH systems in wheat and barley has been emphasized in many reports. A comparative interpretation of the results and conclusions is relatively difficult, due to different environmental conditions, experimental techniques, female genotype (varieties, lines or hybrids), parameters estimated, statistical analysis, etc.

Therefore, the data and the conclusions communicated in this report are valid particularly for our specific experimental circumstances and will be useful for selection of the best pollen sources which can improve the rates of haploid induction in wheat and barley.

CONCLUSIONS

Pollinator genotypes (*Zea mays* L. and *Hordeum bulbosum* L.) influenced significantly the crossability and embryo formation (*in vivo*) in wheat and barley haploid systems.

Bulbosum pollen sources manifested additional obvious indirect (*in vitro*) effects on haploid production efficiency (HPE) in barley.

Barley genotypes and maternal x pollinator interaction effects were manifested only in winter types (six- and two-rowed), while in spring two-rowed types these effects were non-significant.

In barley, the most efficient pollinators (selected for the future haploid production programme) may be considered: Hb2 clone and Hb3 and Hb4 hybrids.

In wheat, Bicaz hybrid (5) was the best pollinator for crossability (S/S and SD/F).

Excepting pollen source 4 (Sv.97), which was significantly less efficient, all the other pollinators gave similar results for embryo formation (E/F).

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Table 1. INFLUENCE OF *BULBOSUM* POLLINATORS ON HAPLOID INDUCTION PARAMETERS

Pollinator	Crossability and embryo formation					
	S/S	SD/F	SD/S	E/F	E/SD	ED/E
	Winter six-rowed					
Hb1	37.6	30.4	77.3	9.5	32.1	55.9
Hb2	42.0	36.2	81.5	14.0	34.9	52.8
Hb3	40.1	33.0	84.5	13.8	35.4	49.0
Hb4	39.6	29.0	69.5	10.4	31.0	43.8
Hb5	36.0	15.4	53.4	3.4	18.3	28.6
	Winter two-rowed					
Hb1	42.2	32.8	71.0	15.9	47.4	44.1
Hb2	42.9	37.9	84.4	17.7	49.7	60.0
Hb3	49.5	40.5	78.8	19.4	47.8	58.5
Hb4	39.5	33.5	84.5	18.4	58.5	63.7
Hb5	47.2	41.9	87.0	15.8	36.8	55.8
	Spring two-rowed					
Hb1	44.2	39.0	83.4	17.7	36.9	40.7
Hb2	-	-	-	-	-	-
Hb3	44.0	34.7	74.7	13.9	36.6	47.0
Hb4	42.1	35.7	80.9	15.3	45.9	59.7
Hb5	49.2	43.6	85.3	7.7	13.4	32.5

* Data from more extensive experiment (41 *vulgare* genotypes)

Table 2. F values for crossability and embryo formation parameters in *H.vulgare* x *H.bulbosum*

Sources of variation	S/S	SD/F	SD/S	E/F	E/SD	ED/E
Winter six-rowed						
Maternal genotype (G)	**2.74**	**3.24***	**2.44**	**3.29***	**4.66***	**1.50**
Pollinator (P)	**0.23**	**2.38**	**3.71****	**2.54***	**1.02**	**1.04**
G x P	**1.20**	**2.03***	**1.44**	**1.32**	**1.00**	**1.67**
Winter two-rowed						
Maternal genotype (G)	**0.53**	**0.67**	**0.39**	**6.00*****	**5.50*****	**2.53***
Pollinator (P)	**0.59**	**0.84**	**0.20**	**0.81**	**1.36**	**1.69**
G x P	**0.31**	**0.56**	**0.20**	**0.79**	**0.56**	**1.43**
Spring two-rowed						
Maternal genotype (G)	**0.94**	**0.80**	**0.78**	**2.82**	**1.25**	**4.76**
Pollinator (P)	**0.27**	**0.57**	**0.92**	**1.87**	**5.05**	**1.99**
G x P	**0.61**	**0.76**	**1.19**	**0.78**	**1.56**	**2.79**

*, **,*** - Significant for P< 0.05, 0.01 and 0.001, respectively

Table 3. HPE ranking of pollinators was generally in accordance with that found for cross-ability:

Barley form	Crossability	HPE
Winter six-rowed	Hb2, Hb3	Hb2, Hb3, Hb4
Winter two-rowed	Hb2, Hb3, Hb4	Hb2, Hb3
Spring two-rowed	Hb1, Hb4, Hb5	Hb2, Hb3, Hb4, Hb5

Table 4. *In vivo* influence of maize pollinators on haploid induction parameters.

Pollinator (mixture)	S/S	SD/F	SD/S	E/F	E/SD	ED/E
A1	77.2	54.6	70.8	17.9	32.7	84.0
A2	82.8	59.1	71.3	16.7	28.2	92.9
Fundulea 102	85.4	65.0	76.1	17.5	27.0	89.3
Suceava 97	78.4	53.4	68.1	15.1	28.3	95.3
Bicaz	93.9	69.6	74.1	6.6	9.4	100

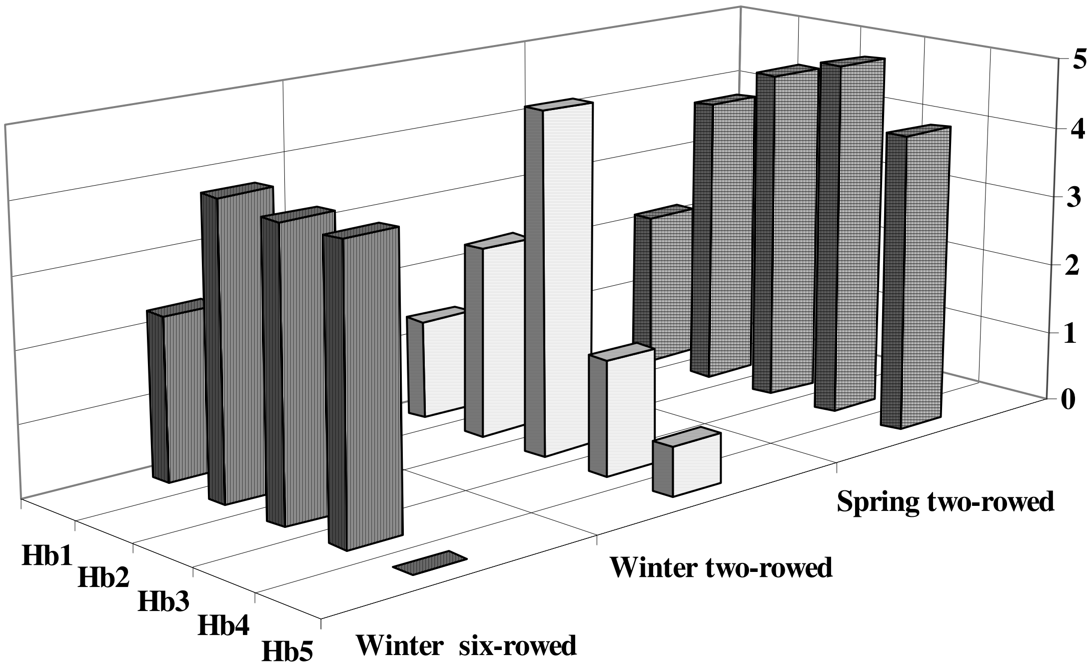


Figure 1. Influence of pollen sources on haploid production efficiency (HPE) in barley (mean of 41 genotypes).

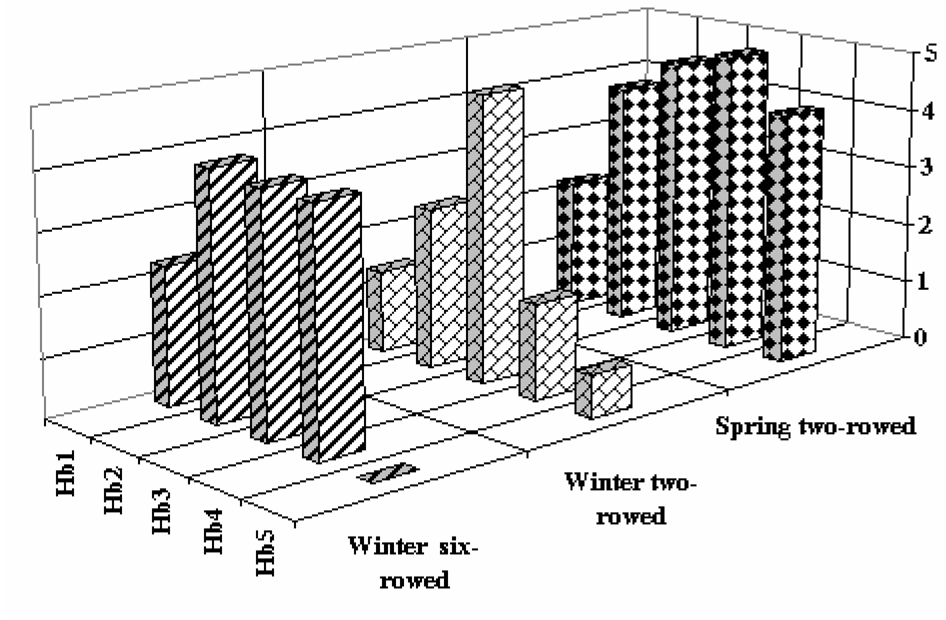
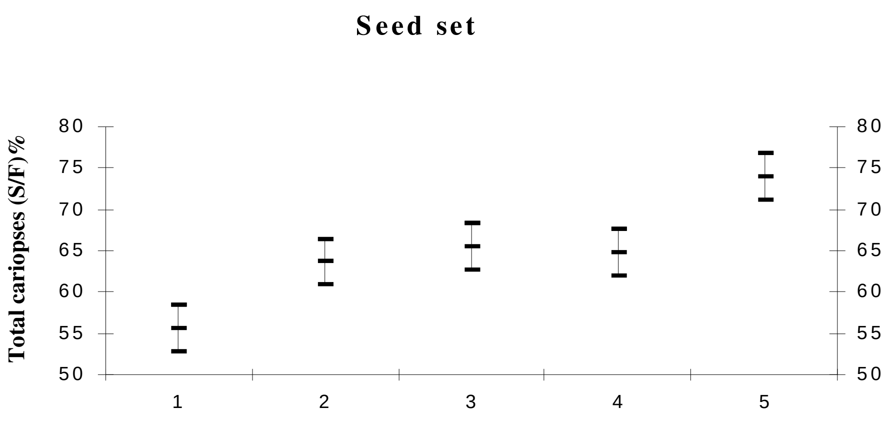
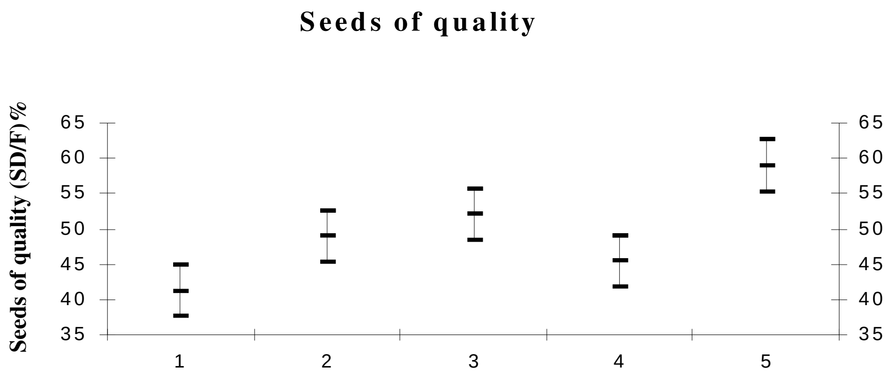
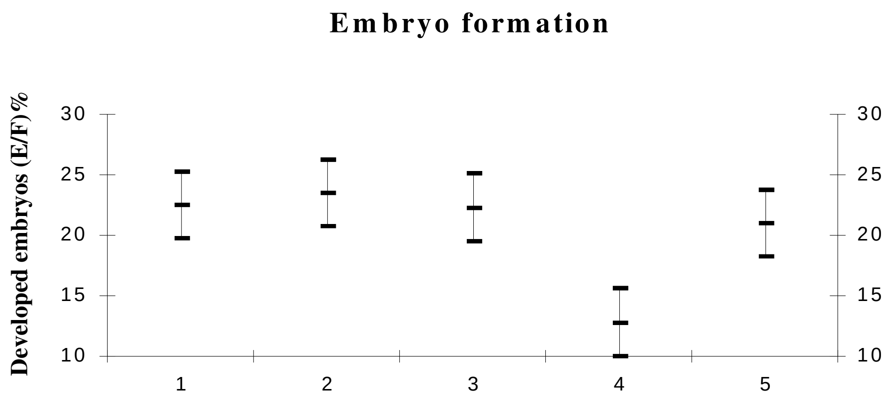


Figure 1. Influence of pollen sources on haploid production efficiency (HPE) in barley (mean of 41 genotypes).

Figure 2. Influence of pollen sources (1,2=mixture; 3=F102; 4=Sv.97; 5=Bicaz) on some efficiency parameters in wheat x maize haploid production.




Seeds of quality
Seeds of quality (SD/F) %
65
60
55
50
45
40
35
1
2
3
4
5


Embryo formation
Developed embryos (E/F) %
30
25
20
15
10
1
2
3
4
5