Haploid regeneration from tetraploid wheat using maize pollen

A. SARRAFI,[1] N. AMRANI, AND G. ALIBERT

Laboratoire de biotechnologie et amélioration des plantes, Équipe d'accueil, Direction de recherche et études doctorales, 832, Institut national polytechnique – École nationale supérieure agronomique de Toulouse, 145, avenue de Muret, 31076 Toulouse, France

Corresponding Editor: G. Scoles

Received March 30, 1993

Accepted September 15, 1993

SARRAFI, A., AMRANI, N., and ALIBERT, G. 1994. Haploid regeneration from tetraploid wheat using maize pollen. Genome, **37**: 176–178.

Crosses were made between 21 tetraploid wheat genotypes (6 parents, 15 F_1 hybrids) and a single F_1 hybrid of maize that was used as the male parent. Plants were grown under controlled greenhouse conditions (daylength, 16 h; temperature, 25°C days and 15°C nights). To enhance embryo survival, 2,4-D (10 mg/L) was applied to spikes 24 h after pollination with maize. Embryos were recovered from the tetraploid wheat genotypes at a rate of 2.34–14.14/100 developed ovaries. Sixty-nine haploid plants were obtained from 3 parents and 12 F_1 hybrids. Fifty-six of these were successfully doubled. General combining ability was significant for the two traits studied, indicating that additive genetic control is important for the number of developed ovaries and haploid embryo production in tetraploid wheat × maize crosses. In this report, we demonstrate the potential of using maize pollen to produce haploid plants from tetraploid wheat genotypes.

Key words: tetraploid wheat, embryo culture, haploid, wheat × maize, combining abilities.

SARRAFI, A., AMRANI, N., et ALIBERT, G. 1994. Haploid regeneration from tetraploid wheat using maize pollen. Genome, **37** : 176–178.

Des croisements ont été faits entre 21 génotypes de blé tétraploïdes, dont 6 parents et 15 hybrides F_1, et un seul hybride F_1 de maïs en tant que parent mâle. Les plantes ont été cultivées en serre sous une longueur de jour de 16 h et une température de 25°C le jour et 15°C la nuite. Pour favoriser la survivance des embryons, les épis ont été traités avec du 2,4-D (10 mg/L), 24 h après la pollinisation avec du pollen de maïs. Les embryons ont été obtenus à partir des génotypes tétraploïdes de blé à un taux variant de 2,34 à 14,14 pour cent des caryopses développés. Soixante-neuf plantules haploïdes ont été obtenues de trois parents et de 12 hybrides F_1; cinquante-six d'entre elles ont été doublées avec succès. L'aptitude générale à la combinaison a été significative pour trois des paramètres étudiés, ce qui indique que le contrôle génétique additif est important pour le nombre de caryopses développés et la production d'embryons haploïdes dans les croisements blé tétraploïde × maïs. Le présent rapport illustre le potentiel de l'emploi du pollen de maïs pour la production de plantes haploïdes à partir de génotypes de blés tétraploïdes.

Mots clés : blé tétraploïde, culture d'embryons, haploïdes, blé × maïs, aptitude à la combinaison.

[Traduit par la rédaction]

Introduction

Double haploid production by means of hybridization between hexaploid wheat (*Triticum aestivum*) and maize (*Zea mays*) is potentially of great value to wheat breeding programs, because it may reduce the time required to achieve homozygosity in breeding lines. In crosses between hexaploid wheat and maize, the maize chromosomes are eliminated from the developing embryo during the initial rounds of cell division (Laurie and Bennett 1986). Maize is not sensitive to the action of dominant genes *Kr1* and *Kr2*, located on the long arms of chromosomes 5B and 5A, respectively (Sitch et al. 1985). These two genes drastically reduce the frequency of fertilization in crosses between hexaploid wheat and *Secale cereale* (Falk and Kasha 1981, 1983).

Previous attempts to cross tetraploid wheat (*T. turgidum* var. *durum*) and maize resulted in embryo formation but not in haploid plant production (O'Donoughue and Bennett 1988).

The importance of additive and dominant genetic effects for embryogenesis and haploid plantlet regeneration has been demonstrated in hexaploid triticale (*Triticosecale* Wittmack) androgenesis by Charmet and Bernard (1984).

In the present study, we conducted an extensive hybridization program involving the pollination of six tetraploid wheat genotypes and their 15 F_1 hybrids with maize to determine the efficiency of haploid regeneration in tetraploid wheat.

Materials and methods

The experiment was carried out with six diverse tetraploid wheat genotypes and their 15 F_1 hybrids. 'Primadur' is a high pasta-cooking quality French cultivar. ENSAT-1 and ENSAT-3 are two pure homozygous lines selected in our department. 'Omrabi' and 'Hourani' are drought-resistant cultivars obtained through the International Center for Agricultural Research in Dry Areas (ICARDA). Verneuil 82-86 is a high-yielding line selected by the Verneuil French Seed Company. Crosses were made between all six genotypes. The maize used as male parent is the F_1 hybrid sweetcorn cultivar 'Seneca 60'. Seeds of all wheat genotypes were sown in a controlled temperature greenhouse (25°C days and 15°C nights). The experimental design consisted of randomized blocks with four replications. Each replication consisted of one pot with three plants. The primary and secondary tillers of each plant were used for crossing procedures. Wheat spikes were emasculated 1–4 days before anthesis. Crosses were conducted with freshly collected pollen and 2,4-D treatment (10 mg/L) was applied as described by Amrani et al. (1993). Approximately 700 florets per wheat genotype were pollinated. Two to three weeks after 2,4-D treatment, embryos were excised under sterile conditions and cultured on commercial Difco Orchid Agar Medium (27.5 g/L), supplemented with 4 g/L sucrose. The cultured embryos were incubated in the dark at

[1]Corresponding author.

TABLE 1. Parental genotypes mean performance and their general combining ability effects (GCA) for embryogenic capacity and haploid plant production in wheat × maize crosses

Genotype	Pollinated		DO/100PF		Em/100DO		HP/100F	Total HP
	Spikes	Florets	Mean	GCA	Mean	GCA		
'Primadur'	34	861	77.21*a*	0.112	14.14*a*	3.848*	1.62	14
ENSAT-1	39	791	74.95*abc*	−0.346	4.55*bcd*	−1.205	0.13	1
ENSAT-3	31	705	75.92*abc*	0.782*	5.14*bcd*	−0.658	0	0
'Omrabi'	35	725	74.15*abc*	−0.566*	3.74*cd*	−1.735*	0	0
'Hourani'	32	650	76.34*ab*	0.214	2.34*cd*	−2.025*	0	0
Verneuil 82-86	34	731	76.63*ab*	−0.196	10.78*ab*	1.775*	0.29	2

NOTE: Means (arc sin $\sqrt{x}$) followed by different letters are significantly different at the $p = 0.05$ level (Newman–Keuls test). DO/100PF, developed ovaries per 100 pollinated florets; Em/100DO, embryo production per 100 developed ovaries; HP/100F, haploid plants per 100 pollinated florets.

*Significant at the $p = 0.05$ level.

20 ± 1°C. When embryos had developed into small seedlings, they were removed and transferred into test tubes containing the same Difco Orchid Agar Medium and placed in a growth chamber under a 16-h daylength at 20 ± 1°C. After 15–20 days, when plants had developed sufficiently, they were transplanted into small pots.

All haploid plants were treated with a 1 g/L colchicine solution for 5 h (Arabi et al. 1991). Doubled haploid plants were grown in the greenhouse and seeds with a high germination capacity were harvested.

The Newman–Keuls test was used for comparing treatment means. General combining abilities were estimated by the fixed model method, No. 3 of Griffing (1956).

Results and discussion

Analysis of variance based on pooled parental and hybrid data for developed ovaries per 100 pollinated florets and embryo production per 100 developed ovaries showed highly significant genotypic effect (data not presented). For the frequency of developed ovaries per 100 pollinated florets, with or without embryos, 'Primadur' and Verneuil 82-86 gave the highest values (77.21 and 76.63, respectively; Table 1). The lowest value was obtained with 'Omrabi' (74.15). For embryo production from developed ovaries (Em/100DO) 'Primadur' and Verneuil 82-86 gave the highest values (14.14 and 10.78, respectively). ENSAT-3 gave an intermediate value (5.14) and 'Hourani' gave the lowest value (2.34).

There was no clear correlation between the production of embryos and the production of developed ovaries. Thus, 'Hourani', which gave a lower yield of embryos (2.34), was highly efficient for production of developed ovaries (76.34). Conversely, 'Primadur' was efficient for both embryo and developed ovary production.

Significant genetic variation for developed ovary production (DO/100PF) as well as embryo formation in tetraploid wheat × maize crosses was observed in this study as has already been reported in hexaploid wheat by Laurie and Reymondie (1991). All 21 tetraploid genotypes studied by us produced embryos upon pollination with maize, in contrast with crosses between tetraploid wheat and *Hordeum bulbosum*, where no embryos were obtained, perhaps because of inhibition by *Kr* gene products (O'Donoughue and Bennett 1988). However, the development of embryos in hexaploid wheat × maize was not always followed by the development of haploid plants (Suenaga and Nakajima 1989). The present experiment indicates that the frequency of embryo to plant conversion depends on the wheat genotype.

From three of six parents and from 12 of 15 F_1 hybrids, a total of 17 and 52 haploid plants were obtained, respectively. Fertile doubled haploids (56 of 69 haploids after colchicine treatment) were grown in the greenhouse. Previous attempts to cross tetraploid wheat and maize resulted in embryo formation but not in haploid plant production (O'Donoughue and Bennett 1988).

As far as the general combining ability (GCA) is concerned (Table 1), most positive and negative values were significant for all the traits studied. One of the six parental lines (ENSAT-3) had a positive value for developed ovaries per 100 pollinated florets (DO/100PF) and 'Primadur' had positive and significant values for embryo formation from developed ovaries. This cultivar should be considered as the best combiner for embryo formation responses. 'Omrabi' is the opposite, with negative GCA values for both traits.

Significant values ($p = 0.05$) for general combining ability (GCA) indicate the importance of additive genetic control for embryogenesis response in tetraploid wheat × maize crosses. The importance of additive genetic effects in androgenesis of hexaploid wheat and hexaploid triticale has also been reported by Bullock and Baenziger (1982) and Charmet and Bernard (1984), respectively.

'Primadur' has the highest GCA values for embryo formation from developed ovaries. Fortunately, 'Primadur' is a French commercial cultivar with a good grain quality and is extensively used in crossing programs in France.

Of the 21 genotypes studied, three parental lines (ENSAT-3, 'Omrabi', and 'Hourani') and three F_1 hybrids ('Primadur' × ENSAT-3, ENSAT-1 × 'Omrabi', and ENSAT-3 × 'Hourani') failed to produce plants; all other genotypes produced a total number of 69 plants. Sixty-nine haploids from three parents and 12 F_1 hybrids were doubled and 56 doubled haploids were obtained and grown in the greenhouse. Viable seeds were harvested from the doubled haploid plants.

Our overall results based on GCA indicate that embryo production could be improved by additive genetic effects in tetraploid wheat × maize, but that haploid plant regeneration frequency is still low and merits further investigation.

Acknowledgement

The authors thank the Regional Council of Midi-Pyrénées for supporting this work (N/Ref. : RECH/9200994).

Amrani, N., Sarrafi, A., and Alibert, G. 1993. Genetic variability for haploid production in crosses between tetraploid and hexaploid wheats with maize. Plant Breed. **110**: 123–128.

Arabi, M.I., Sarrafi, A., Barrault, J., and Albertini, L. 1991. The influence of parental genotype and period of pollination on haploid barley production in *Hordeum vulgare* L. × *H. bulbosum* L. crosses. Cereal Res. Commun. **19**: 405–412.

Bullock, W.P., and Baenziger, P.S. 1982. Anther culture of wheat (*Triticum aestivum* L) F_1'S and their reciprocal crosses. Theor. Appl. Genet. **62**: 155–159.

Charmet, G., and Bernard, S. 1984. Diallel analysis of androgenetic plant production in hexaploid Triticale. Theor. Appl. Genet. **69**: 55–61.

Falk, D.E., and Kasha, K.J. 1981. Comparison of the crossability of rye *Secale cereale* and *Hordeum bulbosum* onto wheat *Triticum aestivum*. Can. J. Genet. Cytol. **23**: 81–88.

Falk, D.E., and Kasha, K.J. 1983. Genetic studies of the crossability of hexaploid wheat with rye and *Hordeum bulbosum*. Theor. Appl. Genet. **64**: 303–307.

Griffing, J.B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Aust. J. Biol. Sci. **9**: 463–493.

Laurie, D.A., and Bennett, M.D. 1986. Wheat × maize hybridization. Can. J. Genet. Cytol. **28**: 313–316.

Laurie, D.A., and Reymondie, S. 1991. High frequencies of fertilization and haploid seedling production in crosses between commercial hexaploid wheat varieties and maize. Plant Breed. **106**: 182–189.

O'Donoughue, L.S., and Bennett, M.D. 1988. Wide hybridization between relatives of bread wheat and maize. Proc. Int. Wheat Genet. Symp., 7th, 1988. pp. 397–402.

Sitch, L.A., Snape, J.W., and Firman, S.J. 1985. Intrachromosomal mapping of crossability genes in wheat *Triticum aestivum*. Theor. Appl. Genet. **70**: 309–314.

Suenaga, K., and Nakajima, K. 1989. Efficient production of haploid wheat *T. aestivum* through crosses between Japanese wheat and maize *Zea mays*. Plant Cell Rep. **8**: 263–266.

How many additional parameters in a conversion model can be evaluated using "corresponding-site" events?

J.R.S. FINCHAM[1]
Division of Biology, University of Edinburgh, Edinburgh, Scotland

Corresponding Editor: A.J.F. Griffiths
Received May 13, 1993
Accepted September 21, 1993

FINCHAM, J.R.S. 1994. How many additional parameters in a conversion model can be evaluated using "corresponding-site" events? Genome, **37**: 179–180.

Contrary to a recent claim by B.C. Lamb and S.A. Zwolinski (1992), the frequencies of "corresponding-site" conversion events (all four chromatids involved in conversion at the same locus in the same meiotic cell) can be used to evaluate only two parameters not otherwise calculable: a coincidence parameter and the frequency of conversion-type events in which a normal 1:1 ratio is restored.

Key words: *Ascobolus immersus*, gene conversion.

FINCHAM, J.R.S. 1994. How many additional parameters in a conversion model can be evaluated using "corresponding-site" events? Genome, **37** : 179–180.

Contrairement à une affirmation récente de B.C. Lamb et S.A. Zwolinski (1992), les fréquences de conversion des sites correspondants (les quatre chromatides étant impliquées dans la conversion au même locus et dans la même cellule méiotique) peuvent n'être utilisées que pour évaluer deux paramètres seulement, qui ne peuvent être calculés autrement, savoir : un paramètre de coïncidence et la fréquence des événements d'un type de conversion dans laquelle un ratio normal 1:1 est rétabli.

Mots clés : *Ascobolus immersus*, gène de conversion.

[Traduit par la rédaction]

Several models for gene conversion, from Holliday (1964) onwards, have been based on single-locus segregation patterns in meiotic octads produced by ascomycete fungi with eight-spored asci. If, as is usually the case, conversion at prophase I of meiosis involves only one chromatid of each chromosome, there are five distinguishable outcomes: 6:2, 5:3, aberrant 4:4 (i.e., overall 4:4 with two mismatched spore pairs), 3:5, and 2:6. There is an invisible sixth outcome, namely, a normal 4:4 ratio, the result either of repair of both chromatids back to their original types or of reciprocal whole-chromatid conversion. The frequencies of these five observable kinds of aberrant asci can be used to evaluate a molecular model for gene conversion that involves no more than five unknown quantities (Fincham et al. 1980). In Holliday's original (1953) model, which postulates reciprocal and symmetrical single-strand exchange, these are the overall frequency of hybrid DNA at the marker locus and four mismatch correction frequencies. Models that, more realistically, allow for asymmetric strand transfer (Meselson and Radding 1975), have too many parameters to calculate from the frequencies of just five classes of aberrant octad.

The simultaneous involvement of two chromatid pairs in conversion at the same locus ("corresponding site events" or double events as I shall call them) can generate four additional easily distinguishable ratios: 8:0, 7:1, 1:7, and 0:8, making nine aberrant classes in all. Lamb and Zwolinsky (1992) have presented an impressive body of data on the frequencies of all nine classes in *Ascobolus immersus*, and, on this basis, claim to be able to evaluate nine parameters in a model allowing either symmetric (reciprocal) or asymmetric (unilateral) DNA strand transfer. One parameter, γ_1, is the fraction of asci with strand transfer at the marked locus, and a second, γ_2, which I will call the coincidence parameter, is the subfraction in which all four chromatids (two chromatid pairs) are involved in such transfer. Thus the overall frequencies of single and double events are γ_1 $(1 - \gamma_2)$ and $\gamma_1\gamma_2$, respectively. Lamb and Zwolinski's other seven parameters relate to the molecular details of the conversion mechanism: α is the probability of formation of asymmetric as opposed to symmetric hybrid DNA at the locus; β is the probability, in the case of asymmetric transfer, that the transferred strand will be wild type rather than mutant; δ is the probability that it has a particular polarity; p, q, r, and s are correction frequencies of the two kinds of mismatches to wild type versus mutant.

It is the purpose of this comment to show that Lamb and Zwolinski's data can be used to evaluate only seven quantities, not nine as they believe. The frequencies of the different octad types, including the double-event classes, are generated by just seven parameters: γ_1, γ_2, and the frequencies of six different outcomes (summing to unity, so only 5 degrees of freedom) of interactions between single pairs of chromatids.

The six possible outcomes of interchromosomal DNA strand transfer at the marked locus between two chromatids are 4:0, 3:1, aberrant 2:2, 1:3, 0:4, and normal 2:2. If only two of the four chromatids present at first meiotic prophase are involved in such interaction, these six outcomes will result in 6:2, 5:3, aberrant 4:4, 3:5, 2:6, and normal 4:4 octads, respectively.

[1]Address for correspondence: 20 Greenbank Road, Edinburgh EH10 5RY, Scotland.

Printed in Canada / Imprimé au Canada