Theor Appl Genet (1984) 67:263–266

Comparisons of alfalfa somaclonal and sexual derivatives from the same genetic source

T. W. Pfeiffer [1] and E. T. Bingham [2]

[1] Department of Agronomy, University of Kentucky, Lexington, KY 40546, USA
[2] Department of Agronomy, University of Wisconsin, Madison, WI 53706, USA

Received June 29, 1983
Communicated by R. Riley

Summary. Tetraploid alfalfa (*Medicago sativa* L.) clones were derived from the same diploid genetic background by four different methods. A phenotypically superior clone was selected from each method and compared for herbage yield and fertility. The four methods and their best clones were: a) In vitro somatic chromosome doubling of one diploid hybrid (HG2-4x); b) selection within a two allele tetraploid synthetic population derived from HG2-4x (HAG); c) somaclonal variant selection from cell suspension culture of the diploid hybrid (NS1); and d) sexual polyploidization of a sibling hybrid (HXG). Clones HG2-4x, HAG, and NS1 were likely diallelic or monoallelic at all loci. Clone HXG was probably tetrallelic or triallelic at most loci. Experiments measured fertility, clonal herbage yield, and herbage yield of test cross progeny for each selected clone. Fertility rankings were HXG = HAG > NS1 > HG2-4x. Clonal herbage yield rankings were HXG = HAG > NS1 > HG2-4x. Test cross progeny herbage yield rankings varied depending on the tester, but, in general, HXG ≧ HAG ≧ NS1 ≧ HG2-4x. Overall the best clones from the sexual methods exceeded the best somaclonal variant which, in turn, was better than the chromosome doubled clone.

Key words: *Medicago sativa* L. – 2n gametes – Tissue culture – Chromosome doubling

Introduction

The possibilities for crop improvement through plant tissue culture have been empasized recently (Larkin and Scowcroft 1981; Shepard et al. 1980; Skirvin 1978; Thomas et al. 1979). Larkin and Scowcroft (1981) conclude that plant cell culture generates genetic variability and that this somaclonal variation is already proving to be of significance for plant improvement. At the same time, novel methods for attempting to exploit the sexual process have also been utilized (Bingham and McCoy 1979; Mendiburu and Peloquin 1977). The normal sexual cycle, which provides genetic recombinants for selection has, however, remained the primary method for crop improvement.

Tetraploid alfalfa (*Medicago sativa* L.) clones have been derived from the same diploid genetic source by four different methods: a) somatic chromosome doubling of one diploid hybrid (Bingham 1980); b) somaclonal variant selection from cell suspension culture developed from the diploid hybrid (Reisch and Bingham 1981); c) selection within a two allele tetraploid synthetic population developed from the doubled diploid hybrid (Pfeiffer and Bingham 1983); and d) sexual polyploidization to produce a tetraploid sibling hybrid (Bingham 1980). The objective of this study was to compare the best clone produced by each method. Fertility, clonal herbage yield, and herbage yield of testcross progeny were measured to indicate the agronomic quality of selected clones.

Materials and methods

Derivation of clones

The origins of the different clones (derived in previous experiments) are illustrated in Fig. 1. Clone HG2-2x is a diploid alfalfa clone bred and selected for its ability to regenerate after suspension culture (McCoy and Bingham 1977). It is a progeny of diploid H275 X diploid GLD 11 and was phenotypically superior to eleven other diploid progenies. Spontaneous doubling of HG2-2x in tissue culture produced the equivalent (or near equivalent) tetraploid HG2-4x. The tissue

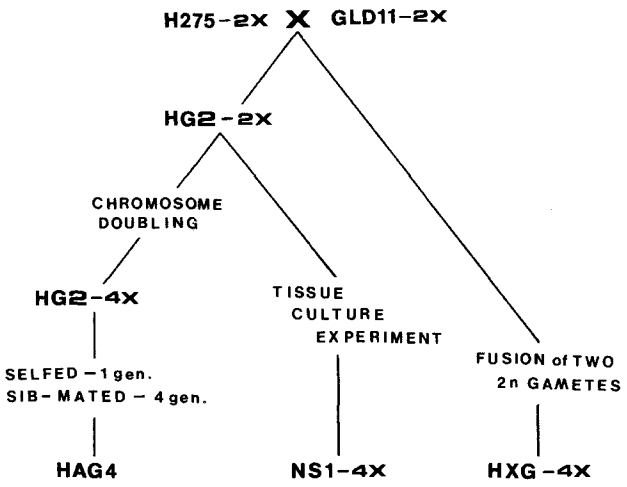

Fig. 1. Development of HG2-2x, its derivatives HG2-4x, NS1, and HAG4 and the related plant HXG-4x

culture derived HG2-4x is typical of more than 100 spontaneously doubled clones of HG2 observed in several experiments. It has recently been shown to be equivalent in vigor and fertility to a colchicine derived HG2-4x (unpublished results). The tissue culture derived HG2-4x was included because it was the clone used to initiate the HAG population, and it has been used as the control tetraploid in the tissue culture work for six years. Additional genetic alterations other than chromosomal doubling in tissue culture from HG2-2x to HG2-4x can not be ruled out entirely.

The autotetraploid HG2-4x was comparatively self-sterile, but 25 progeny were obtained by selfing several thousand flowers. The progeny were sibmated; selection and subsequent sibbing were continued for three additional generations (Pfeiffer and Bingham 1983). This resulted in the HAG4 two allele tetraploid population (coded HAGn-X, n = generation of sibbing, X = numerical identification of selected plant). Assuming no mutation the plants in this population could have a maximum of only two alleles per locus. Selection pressure in the self and first two sib generations was from winter survival and differential fertility; all self and sib seed was bulked and those plants producing more seed contributed more to the next generation. Selection was based on the visual phenotypic vigor of 50 HAG3 plants and the differential fertility of the six selections, which when intermated produced 150 HAG4 plants from which the superior phenotypes were selected. Three different HAGn-X clones were used as representatives because in the first year not enough propagules of any one clone were available for inclusion in all tests.

Clone NS1 was the superior phenotypic selection from an array of 100 somaclonal plants regenerated from HG2-2x after cell suspension culture (Reisch and Bingham 1981). It is a spontaneous variant which was regenerated from an unmutagenized treatment, but possesses a vegetative change resulting in significantly more herbage production than HG2-4x (Reisch and Bingham 1981). The chromosome number based on root-tip squashes is 2n = 32 and pollen mother cell meiosis is typical of HG2-4x and other tetraploids (unpublished observation).

Clone HXG is a tetraploid sexual hybrid resulting from the union of two numerically unreduced gametes (2n gametes) in the cross of H275XGLD11 which produced HG2 (Bingham 1980). It is in effect a sibling of HG2-4x but differs in that four alleles per locus and thus higher order interactions are possible. Clone HXG was phenotypically the best of four sexual tetraploids produced in the cross which produced HG2-2x and is in the same cytoplasm.

Comparisons of clones

Four tests were run to compare the best plant produced by each method in the previous experiments:

1) Male and female cross-fertility data expressed as seeds per flower pollinated were collected in late winter, 1981. Composite pollen from 'Vernal' and two male-sterile clones were used for testing female and male fertility, respectively. A minimum of 50 flowers were pollinated in each combination. No statistical analysis was performed.

2) Open pollinated seed production was analyzed in a field experiment. A single 'Saranac' × 'Vernal' hybrid clone (clone SV) was included as a check. Twelve-week-old cuttings were transplanted on 0.9 m centers 5 June 1981. Pollen plants were interplanted in the plot and honey bee and leaf cutter bee pollinators were stationed nearby. The experiment was a completely randomized design with six replications. Ratings (0 = no seed set to 5 = high seed set) were given independently by three people in September, 1981. Analysis was on the means of the ratings.

3) Cuttings for a clonal vegetative yield study were started 8 March 1981, and transplanted in single row plots 21 May 1981. Plots consisted of three equally spaced (0.3 m) plants with 0.9 m between plots. Plots were harvested, bulking the three plants per plot, on 22 July and 31 August 1981. The planting was completely randomized with five replications and analyzed as a split block with successive observations on the same whole units over a period of time (Little and Hills 1978).

4) Test crosses were made between the selected clones and three tetraploid testers 026-4x, W315-4x, and 6–8 ms. Tester clones 026-4x and W315-4x are unrelated plants produced by colchicine doubling respective diploids; they can have a maximum of two alleles per locus. The male sterile tester, 6–8 ms, is a heterozygous, vigorous clone and could have up to four alleles per locus. Single row plots 1 m long and 0.9 m apart were seeded at the rate of 75 seeds per plot on 15 May 1981. Plots were harvested on 22 July and 31 August 1981. The statistical analysis was the same as for the clonal yield study except replications varied from three to six per cross. Means were analyzed for significant differences within each tester group by the LSD procedure.

Results and discussion

Fertility rankings were the same for both male and female cross fertility; HXG > HAG4-2 > NS1 > HG2-4x (Table 1). This ranking was maintained in open pollinated seed production ratings in the field (Table 2). Clone HXG was not statistically different from the hybrid check, while all other clones were statistically lower in fertility.

In the clonal herbage yield test, all of the experimental clones were lower yielding than the selected Vernal check clone (Table 2). Clone HXG and HAG3-A were not significantly different, but both were superior to NS1 and HG2-4x. Clone NS1 yielded 2.5 times more herbage than HG2-4x, agreeing with previous results (Reisch and Bingham 1981).

Table 1. Female and male cross fertility of the best sexual hybrid (HXG), best two allele synthetic selection (HAG), best tissue culture variant (NS1), and original clone HG2-4x

Clone	Seeds per flower pollinated	
	As female[a]	As male[b]
HXG	5.9	3.0
HAG4-2	5.0	2.2
NS1	3.1	1.5
HG2-4x	0.7	1.0

[a] Composite pollen from 'Vernal' alfalfa used
[b] Results are combined for two different male sterile clones used as testers

Table 2. Open pollinated seed rating and clonal herbage yield of the best sexual hybrid (HXG), best two allele synthetic selection (HAG), best tissue culture variant (NS1), original clone HG2-4x, and two checks

Clone	Op seed rating[b]	Clonal dry wt (g)
Clone SV (check)	5.00a[a]	–
Vernal clone (check)	–	215a
HXG	4.56a	137b
HAG3-A	3.50b	148b
NS1	1.00c	92c
HG2-4x	0.20d	37d

[a] Means followed by the same letter are not significantly different ($P \leqq 0.05$) based on LSD procedure
[b] 0 low – 5 high

Table 3. Mean total herbage yields of progeny of the best sexual hybrid (HXG), best two allele synthetic selections (HAGn), best tissue culture variant (NS1), original clone HG2-4x, and a check. Progeny from crosses to three tetraploid tester plants; two unrelated doubled diploids (026 and W315) and one heterozygous male sterile plant (6–8 ms)

Variety or clone		Tester		
		W315	026	6–8 ms
	Dry wt (g)			
'Vernal' (check)	186	–	–	–
HAG4-1		167a[a]	–	222a
HAG4-2		–	146b	–
NS1		133a	150ab	170b
HG2-4x		104b	132b	156b
HXG		–	184a	192ab

[a] Means within each column followed by the same letter are ot significantly different ($P \leqq 0.5$) based on the LSD procedure. Comparisons made only within each tester group

Herbage yield rankings of the test cross progeny rows varied somewhat depending on the tester except that clone HG2-4x always ranked below the other experimental clones (Table 3). The progeny from 6–8 ms × HAG4-1 were significantly better than the progeny from 6–8 ms × NS1. There was never a significant difference between progeny from HXG and progeny from NS1, although progeny from HXG ranked numerically higher in both cases.

Clones HG2-4x, NS1, and HAGn were all derived from HG2-2x and have a maximum of only two alleles at any locus (assuming no mutations). Such clones are partially inbred at the tetraploid level because of a maximum of only two alleles at any locus (Bingham 1980; Dunbier and Bingham 1975; Mendiburu and Peloquin 1977). Clone HXG, formed by the fusion of a male and a female 2n gamete, potentially carries four alleles per locus; the absolute degree of heterozygosity, however, is dependent on the mechanisms by which the 2n gametes were formed (Bingham 1980; Mendiburu and Peloquin 1977). A high degree of heterozygosity can maximize vigor in a polysomic polyploid such as alfalfa (Bingham 1980; Dunbier and Bingham 1975; Mendiburu and Peloquin 1977) and was the likely cause for high herbage yield in clone HXG (Table 2).

The advanced generation HAGn population was derived from clone HG2-4x by inbreeding and could only decrease in heterozygosity in advanced generations. The improvement in herbage yield and fertility in the advanced generations of HAGn over HG2-4x (Tables 1 and 2) was probably achieved through the accumulation of the most favorable allele at many different loci over generations (Pfeiffer and Bingham 1983).

It has been shown previously (Bingham 1980) and reconfirmed here that the sexual tetraploid hybrid HXG is superior in clonal herbage yield (Table 2) to the tissue culture derived tetraploid HG2-4x. While the best HAGn selections were as good as HXG for clonal herbage yield (Table 2) and herbage yield of progeny from the male sterile tester (Table 3), clone HXG was still superior to the best HAGn selections for fertility (Tables 1 and 2). In alfalfa, fertility responds more to increased heterozygosity than does herbage yield (Dunbier and Bingham 1975).

The genetic mechanism for the tissue culture alteration of HG2-2x to NS1 is not known; however, several traits have been altered and the change is probably more complex than a single gene mutation. Perhaps one or more chromosome substitutions occurred during the polyploidization step similar to the method hypothesized in sorghum (Franzke and Sanders 1964; Simantel and Ross 1963).

In female fertility (Table 1) and clonal yield (Table 2), NS1 exceeded HG2-4x by 4–5 fold and 2.5 fold, respectively. Clone NS1 also exceeded HG2-4x in the ability to donate superior gametes to its progeny (Table 3), indicating that at least some of the culture

derived improvement in NS1 is genetically transmittable. The possibility for culture derived material to be superior to the donor source for certain economic traits has also been reported in sugarcane (Liu and Chen 1978) and potato (Secor and Shepard 1981). For fertility, clonal yield, and progeny yield, NS1 performed similarily or significantly below the level of the best advanced generation HAGn selections (Tables 1–3). Therefore, from the same initial genotype, the sexual cycle, via selfing and four generations of selection and sibbing, has produced plants which exceeded the best variant from one generation of tissue culture. About two years are required to produce and identify the best somaclonal variant and to complete four-five sexual generations. Hence, the time frame is about the same for both methods.

The conclusions of this study are restricted by certain limitations. First, only one initial cross was used to produce the data. Other crosses could provide different results. Second, the sexual and somatic procedures cannot be performed in the same environment or with identical arrays of plants and sample sizes. Consequently, widely different selection intensities were used in the different production procedures. Nevertheless, the high ranking of HXG, even though it was selected from a minimal sample of sexually derived tetraploids, demonstrates the potential value of this method. The best tissue culture variant and the best sexually derived plants were selected from large arrays and their performances provide an initial indication of the potential of the two different methods for producing agronomically improved material.

Acknowledgements. This research was supported by the College of Agriculture and Life Sciences, Madison, WI 53706, and by the SEA of the USDA under Grant No. 5901-0410-8-002 from the competitive Research Grants office.

References

Bingham ET (1980) Maximizing heterozygosity in autopolyploids. In: Lewis WH (ed) Polyploidy: biological relevance. Plenum Press, New York, pp 471–489

Bingham ET, McCoy TJ (1979) Cultivated alfalfa at the diploid level: origin, reproductive stability and yield of seed and forage. Crop Sci 19:97–100

Dunbier MW, Bingham ET (1975) Maximum heterozygosity in alfalfa: results using haploid-derived autotetraploids. Crop Sci 15:527–531

Franzke CJ, Sanders ME (1964) Classes of true-breeding diploid mutants obtained after colchicine treatment of sorghum line, Experimental 3. Bot Gaz 125:170–178

Larkin PJ, Scowcroft WR (1981) Somaclonal variation – a novel source of variability from cell cultures for plant improvement. Theor Appl Genet 60:197–214

Little TM, Hills FJ (1978): Agricultural experimentation: design and analysis. J Wiley and Sons, New York

Liu MC, Chen WH (1978) Tissue and cell culture as aids to sugarcane breeding. 2. Performance and yield potential of callus derived lines. Euphytica 27:273–282

McCoy TJ, Bingham ET (1977) Regeneration of diploid alfalfa plants from cells grown in suspension culture. Plant Sci Lett 10:59–66

Mendiburu AO, Peloquin SJ (1977) The significance of 2n gametes in potato breeding. Theor Appl Genet 49:53–61

Pfeiffer TW, Bingham ET (1983) Improvement of fertility and herbage yield by selection within two-allele populations of tetraploid alfalfa. Crop Sci 33:633–636

Reisch B, Bingham ET 1981 Plants from ethionine-resistant alfalfa tissue cultures: variation in growth and morphological characteristics. Crop Sci 21:783–788

Secor GA, Shepard JF (1981) Variability of protoplast-derived potato clones. Crop Sci 21:102–105

Shepard JF, Bidney D, Shahin E (1980) Potato protoplasts in crop improvement. Science 208:17–24

Simantel GM, Ross JG (1963) Colchicine-induced somatic chromosome reduction in Sorghum. 3. Induction of plants homozygous for structural chromosome markers in four pairs. J Hered 54:277–284

Skirvin RM (1978) Natural and induced variation in tissue culture. Euphytica 27:241–266

Thomas E, King PJ, Potrykus I (1979) Improvement of crop plants via single cells in vitro – an assessment. Z Pflanzenzücht 82:1–30