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Transmission and yield effects of a gibberellin mutant allele in potato

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Summary

A selected clone of *Solanum tuberosum* Group Andigena PI 347773 (clone Adg 11.1) and the cultivar Superior had previously been shown to be heterozygous at a gibberellin related dwarfing locus, ga_1. Experiments were conducted to test for gametophytic selection for alleles at the ga_1 locus in reciprocal crosses and for the effect of genotype on tuber yield. The genotypes of individuals from Superior-Adg 11.1 reciprocal F_1 families were determined by test crossing. Genotypic classes could be distinguished by the percentages of dwarf seedlings in their test cross progeny. No differences were found in the genotypic distributions of the reciprocal families, which indicates gametophytic selection at this locus is not the cause of reciprocal yield differences. Superior-Adg 11.1 individuals classified by ga_1 locus genotype were evaluated for tuber yield. Tuber yields were highly correlated with genotype in both reciprocal families. Yields followed the pattern of simplex > duplex > triplex, indicating that the recessive mutant ga_1 allele exhibited a dose effect stimulating yield.

Introduction

Gibberellins have been found to inhibit tuber formation in potatoes (Vince-Prue, 1985), and break or shorten tuber dormancy (Coleman, 1987). Driver (1943) suggested that the effects of photoperiod on tuberization and yield might be brought about by hormonal activity stimulated in the foliage. This has been supported by subsequent work relating photoperiod, gibberellic acid (GA) activity and tuberization.

Batutis & Ewing (1982) determined that Group Tuberosum is induced to tuberize when long nights are not interrupted, and that this induction is regulated by the photo-sensitive pigment phytochrome. Induction to tuberize has been shown to be correlated with a reduction in GA activity and cessation of flowering (Krauss & Marschner, 1982). It has long been noted that the tuberization "promoter(s)" can be transferred across grafts from "induced" potato leaves to "non-induced" buds (Gregory, 1956). In addition to tuberization, maturity and final tuber yield have also been shown to be determined by the scion (Trudgill & Thompson, 1987).

Sanford & Hanneman (1982) were the first to specifically relate GA status to observed differences for yield in exact reciprocal families within *S. tuberosum*. Their

work with Group Tuberosum cultivars and Group Andigena indicated that large reciprocal yield differences depend on the mating of clones of opposite maturity extremes (i.e. early × late and reciprocal). It was noted that maturity in this case must be precisely defined since families could differ greatly in their abilities to set tubers and produce yield with no detectable differences in vine appearance, flowering or time of senescence. This may not be too surprising since such traits as rosette growth habit and flowering, although very closely associated developmentally in some species, have been shown to be controlled by different genes and physiological processes (Vince-Prue, 1985).

While the parents of Sanford & Hanneman's (1982) reciprocal crosses had different species' cytoplasm, this was assumed to not be a critical factor since some reciprocals in these cytoplasms do not demonstrate yield differences (Tarn & Tai, 1977), while such yield differences can be demonstrated within a cytoplasm (Lazin & Ewing, 1979). The work of Staub et al. (1982) indicated that reciprocal differences were generally more dependent on the genes of the parents used than on cytoplasm, and that maternal rather than cytoplasmic effects appeared to be influential. Simple cytoplasmic effects also fail to be in accord with the observed reduction of the reciprocal effect in F_2 families (Sanford & Hanneman, 1982).

In vitro pollen germination and pollen tube growth have been reported to be greatly affected by GA in some species (Bose, 1959; Carmichael, 1970; Chandler, 1958; Stowe & Yamaki, 1957).

Both the *S. tuberosum* cultivar Superior and Adg 11.1, a clone selected from Gp. Andigena PI 347773 have been reported to be heterozygous for a dwarfing gibberellin deficiency which is apparently conferred by the nulliplex (completely recessive) condition at a single locus, the ga_1 locus (Bamberg & Hanneman, 1991). Superior is triplex (GA_1 GA_1 GA_1 ga_1), and Adg 11.1 is simplex (GA_1 ga_1 ga_1 ga_1). These parents also produce particularly large and consistent reciprocal differences in tuber yield (Bamberg & Hanneman, 1991; Sanford & Hanneman, 1982).

The present work was done to determine if gametophytic selection based on the ga_1 locus could explain the large tuber yield differences previously noted in reciprocal families of Superior and Adg 11.1. The theoretically necessary elements were in place, namely a plausible basis for selection and differences upon which to select. As already noted, GA has been shown to influence both tuber yield and pollen tube growth (the basis for selection), and the parents used are both heterozygous (produce variable gametes) with respect to a gibberellin determining locus (differences upon which to select). If gametophytic selection based on the ga_1 locus determines reciprocal yield differences, the higher yielding reciprocal families must have a greater proportion of particular non-dwarf genotype(s) which confer greater tuber yield. This would require Ga_1 alleles to be less than completely dominant, having a dose effect which causes different average tuber yields among the various non-dwarf genotypes (simplex, duplex, triplex, quadruplex).

Materials and methods

Reciprocal families were produced by crossing Superior × Adg 11.1 (family "A") and Adg 11.1 × Superior (family "B"). This cross had been previously shown to exhibit large and consistent reciprocal yield differences. Seeds of these two families were planted and 72 non-dwarf (Ga_1 – – –) phenotype individuals were selected

at random from each. These were planted in the field at the University of Wisconsin Agricultural Research Station at Sturgeon Bay. Pistils from each clone were pollinated with simplex Adg 11.1 pollen using the cut-stem technique in an air-conditioned greenhouse (Peloquin & Hougas, 1959). This was done so that the percentage of dwarfs in the resulting progeny could be determined, thereby revealing the genotypes of the pistillate parents. A true testcross would have employed a nulliplex tester, but this was not done because it is relatively difficult to induce dwarfs to produce sufficient pollen and flowers for crossing.

Since the genotypes of the parents were known, genotypic arrays in the hybrid families could be predicted. Triplex Superior (GA_1 GA_1 GA_1 ga_1) was expected to produce ½ (Ga_1 ga_1) and ½ (GA_1 GA_1) gametes, and simplex Adg 11.1 (GA_1 ga_1 ga_1 ga_1) was expected to produce ½ (Ga_1 ga_1) and ½ (ga_1 ga_1) gametes. Thus, if random unions of these gametes produced both of the hybrid families, the ratio of their genotypes was expected to be about ¼ triplex (GA_1 GA_1 GA_1 ga_1), ½ duplex (GA_1 GA_1 ga_1 ga_1), and ¼ simplex (GA_1 ga_1 ga_1 ga_1) regardless of the direction of the cross. Each of these three possible genotypes could be distinguished by the percentage of nulliplex dwarfs in the progeny resulting from test crosses with simplex Adg 11.1, as follows: it was expected that the simplex clones would produce 3 / 6 = 50% double recessive gametes, the duplex clones would produce 1 / 6 = 16% double recessive gametes, and the triplex clones would produce 0 / 6 = 0% double recessive gametes. The Adg 11.1 tester was previously determined to be simplex, so was known to produce 3 / 6 = 50% double recessive gametes. Thus, when using this tester, dwarf frequencies in the progeny of the three genotypic classes (simplex, duplex, triplex) was expected to be one-half of their expected frequency of double recessive gametes. Therefore, a simplex clone crossed with simplex Adg 11.1 tester was expected to produce 25% dwarfs, a duplex clone 8% dwarfs and a triplex clone 0% dwarfs. Previous work demonstrated that triplex × simplex actually produces dwarfs at a rate of about 1%, presumably by double reduction (Bamberg & Hanneman, 1991), so this was set as the expected dwarf frequency in progeny of triplex clones. Similarly, it was recognized that dwarf frequencies in progeny of other genotypic classes could vary slightly from expectations due to double reduction.

About 50 of the clones from each reciprocal family produced sufficient test cross seeds for analysis. In the following spring, these seedlots were pretreated with 1500 ppm GA_3 for 18 hr and sown in 7.5 cm (3 inch) clay pots. Each lot was transplanted to soil in two flats containing 54 plants each. At about 6 weeks post-transplanting, dwarfs were easily distinguished from normal seedlings by their short stature, short internode length and dark green color. The number of dwarfs in each family was tallied and their percentage calculated.

The percentage of dwarfs in the test cross progeny of a given clone was used to assign the genotype to that clone. As explained above, if union of gametes was random (no gametophytic selection) both reciprocal families would be expected to to have genotypic ratios of ¼ simplex:½ duplex:¼ triplex, with test cross progeny dwarf percentages of 25%, 8%, and 1%, respectively. Two questions were to be answered from the resulting data: 1) were the genotypic arrays of the reciprocal families different?, and 2) did genotypic arrays differ from the 1 simplex:2 duplex:1 triplex ratio predicted by random unions of gametes? The first question was answered by comparing the distributions of dwarf percentages in the two reciprocal families with the Kolmogorov-Smirnov test (Steele & Torrie, 1980). Whether the

genotypic arrays differed from 1:2:1 was answered as follows: clones were assigned genotypes with the assumption of no gametophytic selection. Thus, the quarter of the population with the highest percentage of progeny dwarfs were classified as simplex, the quarter of the family with the lowest progeny dwarf percentages were classified as triplex, and the remaining half of the family with intermediate dwarf percentages were classified as duplex. Clones were not divided exactly in a 1:2:1 ratio, since all clones with identical dwarf percentages were placed in the same class. The mean observed progeny dwarf percentages were then calculated and compared to the expected progeny dwarf proportions, namely, for simplex parents = 25 %, for duplex parents = 8 %, and for triplex parents = 1 %. In addition, the confidence intervals for progeny dwarf percentages expected from a given genotype were calculated. Clones were placed in genotypic classifications according to these ranges. The resulting ratio of genotypic classes was compared to 1:2:1 by Chi square calculations.

Clones assigned genotypes under the assumption of a 1:2:1 ratio of triplex, duplex, and simplex clones, respectively were made into a balanced bulk for each of the three genotypes. These became the treatments in a randomized complete block yield trial with two replicates at the University of Wisconsin Agricultural Research Station at Hancock. Plants were spaced at 60 cm (2 ft) in rows 1.2 m (4 ft) apart.

Adg 11.1 × Superior genotypes were evaluated in 1986. Tubers were harvested 117 days after planting (planting date June 12) in the field. Superior × Adg 11.1 genotypes were evaluated in 1987 in an identical design with tubers harvested 90 days after planting in the field (planting date June 10). The data were analysed by analysis of variance (ANOVA) as separate experiments for each reciprocal family. The LSD values were calculated to determine which treatment means were significantly different.

Results and discussion

The distributions of dwarf percentages represented by reciprocal families A and B were not significantly different. To claim that the two distributions were different at only the $P = 0.20$ level, a critical value of 14 is needed in the Kolmogorov-Smirnov test, but the calculated statistic for these distributions was only 4.

Tables 1a and 1b present the progeny dwarf percentages, where parental clones are classified as triplex, duplex or simplex by dividing them into lowest, middle, and highest progeny dwarf percentages in a 1:2:1 ratio. Values of individual clones varied considerably from the expected 1 %, 8 % and 25 % values, but means of the genotypic classes fit very well with expectations and were quite similar in both of the two reciprocal families.

The departure of individual clones from expected progeny dwarf percentages could come from two sources: the chance variation of observed percent dwarfs from a random sample assayed with absolute accuracy, and factors affecting relative sprouting and survival of dwarf and normal seedlings, resulting in inaccuracy of the observed dwarf frequencies.

Limits of chance variation in samples observed with absolute accuracy can be set by determining the 95 % confidence intervals of percent data. These limits for 1 %, 8 % and 25 % are 0 – 4 %, 4 – 15 %, and 17 – 34 %, respectively (Steele & Torrie, 1980). In the 1:2:1 genotypic classification shown in Table 1a and 1b, only a few clones would be reclassified to better fit into these confidence intervals. The result

Table 1a. Test crosses of (Superior × Adg 11.1) clones with Adg 11.1 – seedling dwarf frequencies and corresponding genotypic distribution.

Low ¼ of family (13/53 = 25% = triplex)		Middle ½ of family (25/53 = 47% = duplex)		High ¼ of family (15/53 = 28% = simplex)	
clone	% dwarfs	clone	% dwarfs	clone	% dwarfs
A14	0	A06	4	A03	15
A25	0	A54	4	A04	15
A31	0	A08	5	A57	15
A48	0	A11	5	A65	15
A59	0	A15	5	A71	15
A70	0	A44	5	A20	16
A38	1	A53	5	A72	17
A49	1	A10	6	A36	20
A66	1	A21	6	A60	21
A05	2	A32	6	A16	23
A13	2	A35	6	A07	24
A34	2	A18	7	A43	25
A22	3	A41	7	A37	26
		A61	7	A39	26
mean	1	A68	7	A56	34
expected	1	A69	7		
		A23	8	mean	20
		A30	8	expected	25
		A45	8		
		A58	8		
		A62	8		
		A42	9		
		A55	9		
		A63	13		
		A12	14		
		mean	7		
		expected	8		

is a triplex:duplex:simplex genotypic ratio of 13:30:10 for family A, and 15:25:12 for family B. Chi square values gauging the difference of these proportions from the hypothesized 1:2:1 give P values of 0.53 and 0.83, respectively (both non-significant).

From the above analyses, it was concluded that clones in reciprocal families have similar distributions of test cross progeny dwarf percentages. This implies that ratios of genotypes with respect to the dwarf locus are similar in both reciprocal families, which precludes gametophytic selection as the basis of reciprocal yield differences. Lack of gametophytic selection leads to a prediction that genotypes of clones will be in a 1 triplex:2 duplex:1 simplex ratio in both reciprocal families, and observed distributions of progeny dwarf percentages support such a classification.

Table 2 presents the results of yield trials on individuals classified by cross direction (Superior × Adg 11.1 and reciprocal), and genotype, with respect to the ga_1

Table 1b. Test crosses of (Adg 11.1 × Superior) clones with Adg 11.1 – seedling dwarf frequencies and corresponding genotypic distribution.

Low ¼ of family (12/52 = 23% = triplex)		Middle ½ of family (26/52 = 50% = duplex)		High ¼ of family (14/52 = 27% = simplex)	
clone	% dwarfs	clone	% dwarfs	clone	% dwarfs
B03	0	B18	3	B30	13
B06	0	B39	3	B56	13
B31	0	B42	3	B34	17
B32	0	B21	4	B38	17
B53	0	B41	4	B58	18
B08	1	B12	5	B20	19
B13	1	B05	6	B35	21
B23	1	B11	6	B10	22
B71	1	B22	6	B07	23
B72	1	B49	6	B02	25
B48	2	B70	6	B43	25
B55	2	B15	7	B14	29
		B37	7	B14	31
mean	1	B45	8	B54	31
expected	1	B24	9		
		B57	9	mean	22
		B60	9	expected	25
		B04	10		
		B16	11		
		B36	11		
		B63	11		
		B66	11		
		B69	11		
		B25	12		
		B29	12		
		B62	12		
		mean	8		
		expected	8		

locus (according to test cross results; see Tables 1a, 1b).

Each genotype within both of the reciprocal families exhibited significantly different tuber yields. In both cases yields followed the pattern simplex > duplex > triplex. These reciprocal families were tested and analysed in separate experiments, but this is of no consequence since comparisons of interest are only among genotypes within families. Plots in 1986 yielded more than those in 1987, but the relationship of yields among genotypes remained very similar in both years. It had been previously established that these families consistently exhibit large reciprocal differences.

The work described here tests the hypothesis that reciprocal gametophytic selection combined with differing yields for genotypes with respect to the ga_1 locus are

Table 2. Average per-hill yields of different genotypic classes within Superior – Adg 11.1 reciprocal families.

a. Adg 11.1 × Superior – 1986 Hancock Trial[1]	
Adg 11.1 × Superior genotype	Average per hill wt (gm)
simplex	1467 a
duplex	1179 b
triplex	998 c
b. Superior × Adg 11.1 – 1987 Hancock Trial[2]	
Superior × Adg 11.1 genotype	Average per hill wt (gm)
simplex	1005 a
duplex	903 b
triplex	510 c

[1] Different letters indicate significantly different yields at $P = 0.05$ ($lsd_{0.05} = 142$). CV = 2.7%.
[2] Different letters indicate significantly different yields at $P = 0.05$ ($lsd_{0.05} = 34$). CV = 1%. These yields were lower than those of the reciprocal family, as the plants were grown in different years and harvested after 90 or 117 days.

the basis of the mean tuber yield differences observed in these reciprocal families. The results obtained do not support gametophytic or any other form of reciprocally different selection on the ga_1 locus.

The clear dose effect of ga_1 alleles on tuber yield, however, is intriguing. As previously stated, seedlings appear to exhibit only two phenotypes, dwarf and normal. Even when non-dwarf clones were progeny tested to determine their genotypes, and those genotypes bulked into separate plots, no obvious differences in flowering, vine characteristics or maturity were seen. It should be noted however, that no attempts were made to objectively quantify these parameters. There is good reason for doing so now that differences in tuber yield have been demonstrated.

This dose effect can be described as a stimulation of tuber yield by ga_1 alleles, but perhaps more correctly as a depression of tuber yield by Ga_1 alleles, since hybrids with more gibberellin producing alleles (Ga_1) may favor vine growth, and those with less of these alleles, may favor tuber development. Furthermore, this effect must be hypostatic to a condition created in the hybrids which is not present in the parents, since the *tuberosum* (Superior) parent is high yielding despite being triplex, and the *andigena* (Adg 11.1) parent is low yielding, despite being simplex. Whatever the explanation, to the authors' knowledge, this is the first report of a specific single allele with a clear dose effect on tuber yield.

This research has demonstrated that reciprocal yield differences are not based on gametophytic selection on the ga_1 locus. However, considering the dose effects exhibited, this locus may be a useful tool for further study of the the role of gibberellin on tuber initiation and development.

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