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Inheritance of supernodulation in soybean and estimation of the genetically effective cell number

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Summary. Provided the nature of inheritance is known, the frequency of homozygous mutant plants in individual M_2 families (derived from M_1 seed) can be used to estimate the genetically effective cell number (GECN). Segregation ratios in M_3 families derived from M_2 wild-type plants indicated that the supernodulation characters *nts*382, *nts*1007 and *nts*183 are inherited as Mendelian recessives. The nature of inheritance was also known or confirmed to be recessive by crossing the wild type to these and several other mutants derived from the same population of M_2 families. Subsequently, using the frequency of mutant plants in individual M_2 families, the GECN for soybean was calculated to be approximately two.

Key words: Inheritance – Supernodulation – Mutagenesis – Soybean – Genetically effective cell number (GECN)

Introduction

Nodulation mutants have been reported in several legume species. In soybean, the nature of inheritance in four naturally occurring variants has been documented. Mutant allele rj_1 is recessive and conditions non-nodulation (Williams and Lynch 1954), whereas Rj_2, Rj_3 and Rj_4 are dominant and responsible for strain-dependent ineffective nodulation (Caldwell 1966; Vest 1970; Vest and Caldwell 1972). Other mutations affecting nodulation in soybean have recently been induced by mutagenesis in the parent cv Bragg and these include supernodulation, such as *nts*382, *nts*1007 and *nts*183, and additional non-nodulating variants *nod*49, *nod*772 and *nod*139 (Carroll et al. 1986). The 15 supernodulating mutants described by Carroll et al. (1985a, b) can nodulate in the presence of soil nitrate and are defective in autoregulation, the mechanism by which nodule number is controlled by the legume host. Because these mutants nodulate in the presence of nitrate, they have been designated *nts* for nitrate-tolerant symbiosis.

With the exception of *nod*49, these supernodulating and non-nodulating mutants were derived from individual segregating M_2 families, and therefore arose from separate mutation events (Carroll et al. 1985a, 1986). Provided the nature of inheritance is known, the frequency of the mutant plants in individual M_2 families can be used to estimate the genetically effective cell number (GECN) (Li and Redei 1969). This is the number of germline cells in the mutagenized M_1 seed that give rise to a segregating M_2 family. The GECN is of practical significance in the planning of a mutagenesis and selection program for a particular species, since each germline cell in the seed represents a genetic unit. The value of the GECN is considered to range from 1–10, depending on the species (Redei 1975). Where the GECN is greater than one, a chimeric plant results from the M_1 seed.

Traditionally, the nature of inheritance of characters is ascertained from the F_1 phenotype and F_2 segregation ratios derived from crosses between pure-breeding mutant and wild-type lines. In this paper, an additional approach is described. M_2 families (derived from mutagenized M_1 seed) segregated for the supernodulation phenotype and contained both supernodulating mutants and wild-type siblings. If the supernodulation character is inherited as a Mendelian recessive, M_2 plants heterozygous for the mutated gene would ex-

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press the wild-type phenotype. However, M_3 progeny derived from self-fertilization of such plants would segregate 1 mutant to 3 wild types. In analogy to traditional crossing methods, M_2 heterozygous plants and their resultant M_3 progeny are equivalent to F_1 and F_2 generations, respectively. Using this approach, M_3 progeny derived from M_2 mutant and wild-type siblings were screened for segregation of the supernodulation character. This genetic analysis was confirmed by crossing these and other supernodulating mutants with wild-type soybean. The segregation ratio of the mutant characters in their respective M_2 families was then used to estimate the GECN in soybean.

Materials and methods

Following mutagenesis (Carroll et al. 1986), M_2 families of seeds were randomly sampled from individual M_1 plants. M_2 families 382, 1007 and 183 were 3 of 15 families that segregated for the supernodulation phenotype (Carroll et al. 1985a). Both mutant and wild-type siblings were saved to produce M_3 families (i.e. families derived from single M_2 plants). True breeding stability of the mutant characters was demonstrated in M_3 progeny of M_2 mutant selections (Carroll et al. 1985a), and those M_3 families derived from wild-type M_2 plants were screened for segregation of the supernodulation character. The plants were cultured and inoculated with *Bradyrhizobium japonicum* strain USDA110 or CB1809 as described by Carroll et al. (1985a). Potassium nitrate (5 m*M*) was administered to the pots since this accentuated the difference observed between mutant and wild-type plants. Parent CV Bragg and wild-type segregant siblings of supernodulation mutants were indistinguishable for nodulation in the presence of nitrate, and therefore nitrate-grown Bragg plants were generally not included in screening experiments. However, as a N_2-dependent control, Bragg plants were cultured as described above, except that these pots received N-free nutrient solution. Plants were screened for nodulation phenotype, and measurements were made of nodule number, nodule fresh weight, nitrogenase (acetylene reduction) activity and plant fresh weight. The acetylene reduction technique was the same as that used by Carroll et al. (1985a). Chi-square analysis was used to statistically test segregation ratios. It was necessary to include the Yates correction term in Chi-square calculations due to the size of the expected classes (Strickberger 1976).

The nature of inheritance was confirmed in these supernodulating lines and in other similar lines (*nts*733, *nts*2062 and *nts*501) using traditional crossing methods with plant material grown with nitrate under the same conditions as described above. Subsequent to determining the nature of inheritance, the frequency at which homozygous mutants were originally identified in the segregating M_2 family was used to estimate the GECN in the progenitor M_1 seed. When the germline is composed of 1 cell at the time of mutagenesis, recessive mutations will segregate in the M_2 generation at the simple Mendelian ratio of 1 mutant to 3 wild types. If GECN = 2, recessive mutations will segregate at a frequency of 1 mutant to 7 wild types (i.e. 1 to 3 + 0 to 4) in the M_2. If GECN = 3, the segregation ratio will be 1 to 11 (i.e. 1 to 3 + 0 to 4 + 0 to 4), etc. (Redei 1975). Thus, the frequency of homozygous recessive mutants can be used to calculate the apparent GECN as follows: GECN = t/4a, where t = total no. of plants in a specific M_2 family, and a = total no. of homozygous mutants in a specific M_2 family.

Table 1. Segregation ratios of M_3 progeny derived from wild-type plants in families 1007, 382 and 183

Family	Frequency of segregating M_3 families[a]	Segregation ratio in M_3 families *nts*: wild-type[b]	Chi-square[c] 1:3
1007	3/23	3:13	0.17
		12:28	0.31
		7:24	0.01
382[d]	1/6	14:43	0.00
183	1/1	8:15	0.71

[a] Frequency of segregation in M_3 families derived from M_2 wild-type plants; numerator = no. of segregating M_3 families, denominator = total no. of M_3 families screened

[b] Segregation ratios in individual M_3 families derived from M_2 wild-type plants

[c] Chi-square calculated from the observed segregation ratios and the expected segregation ratio expected for simple Mendelian monogenic recessive inheritance. Chi-square (0.05 level of significance; 1 degree of freedom) = 3.84. Therefore, chi-square values listed in the table are not significant

[d] Cited from Carroll et al. (1985b)

Results

Inheritance of nts1007, nts382 and nts183

Like all the M_2 families segregating for supernodulation (Carroll et al. 1985a), M_2 families 1007, 382 and 183 segregated for two nodulation phenotypes: supernodulation and wild-type. All M_3 progeny derived from self-fertilized supernodulating 1007, 382 and 183 mutant plants were supernodulating. These results indicated that supernodulating mutants in these M_2 families were homozygous for respective mutations, and this was consistent in all of the other supernodulating mutant families described previously (Carroll et al. 1985a). In contrast, not all M_2 wild-type plants in these families were pure-breeding. In family 1007, 3 out of 23 wild-type derived M_3 families segregated for the supernodulation character (Table 1). In selected line 382, one of six M_3 families segregated for the mutant character (Table 1). Only one M_2 wild-type in family 183 produced M_3 progeny, and this progeny segregated for the supernodulation phenotype (Table 1).

The mutant to wild type ratio in segregating M_3 families derived from M_2 wild types closely approximated 1 mutant to 3 wild types (Table 1). This is the expected segregation ratio for a Mendelian monogenic recessive in progeny derived from a self-fertilized heterozygous plant. Therefore, the *nts*382, *nts*1007 and

*nts*183 phenotypes appear to have resulted from monogenic recessive mutations. As reported for progeny derived from all the original mutant selections (Carroll et al. 1985a), mutant segregants were characterized by substantially increased nodule number and nitrogenase activity (Table 2) per plant when grown on nitrate. For comparison of nodulation parameters, N_2-dependent wild-type Bragg plants were harvested with 1007 segregants that were cultured on nitrate. The root systems of these plants are shown in Fig. 1. The pattern of nodulation in 1007, 382 and 183 supernodulating segregants was very similar. Mutant segregants had significantly more nodules on the tap root and especially on lateral roots, clearly contrasting the pattern of nodulation in both N_2-dependent wild-type Bragg and nitrate-grown wild-type siblings in which autoregulation is operative (Fig. 1). Consistent with the trend reported earlier, these supernodulating segregants had significantly lower plant fresh weights than did wild-type siblings (Table 2).

Table 2. Symbiotic properties of supernodulating and wild-type siblings in segregating M_3 families of 1007, 382 and 183. The plants were harvested 49 days (1007) and 51 days (382 and 183) after planting and culture on 5 m*M* KNO_3 as described in "Materials and methods". All seeds were planted on the same day and were inoculated with *Bradyrhizobium japonicum* strain USDA110 at day 0 and day 4. Each entry in the table is the mean ± SE of 4–18 plants

Family	Segregant phenotype	
	Supernodulating	Wild-type
	Nodule no. per plant	
1007	621 ± 38	19 ± 3
382	789 ± 49	32 ± 5
183	1043 ± 94	70 ± 8
	Nitrogenase activity (nmol $C_2H_4 \cdot plant^{-1} \cdot min^{-1}$)	
1007	90 ± 10	11 ± 3
382	56 ± 12	16 ± 4
183	91 ± 22	29 ± 11
	Plant fresh weight (g)	
1007	8.9 ± 0.7	13.9 ± 0.9
382	8.2 ± 0.6	15.2 ± 0.8
183	12.3 ± 0.8	16.5 ± 1.6

The recessive mode of inheritance in *nts*382, *nts*1007 and *nts*183 was confirmed by traditional backcrossing to the wild-type cv Bragg, which always yielded a wild-type phenotype in the F_1 progeny. Similar crosses showed that *nts*733, *nts*2062 and *nts*501 were also inherited as Mendelian recessives. Genetic analysis and complementation studies on this material are described in detail in Delves et al. (1988). Crosses of *nts*382 with cvs Clark and Williams gave F_2 segregation ratios of 8 supernodulating to 22 wild-type and 8 supernodulating to 28 wild-type plants, respectively. Mutant *nts*501 crossed with cv Clark gave ratios of 8:35 supernodulating to wild-type F_2 segregants. This confirmed the recessive Mendelian inheritance in genotypes other than those from which the mutant lines were derived. F_3 seed was collected from F_2 supernodulating segregants of these crosses and grown to check the supernodulation phenotype. Two hundred seeds were grown from each of the above crosses and all plants showed

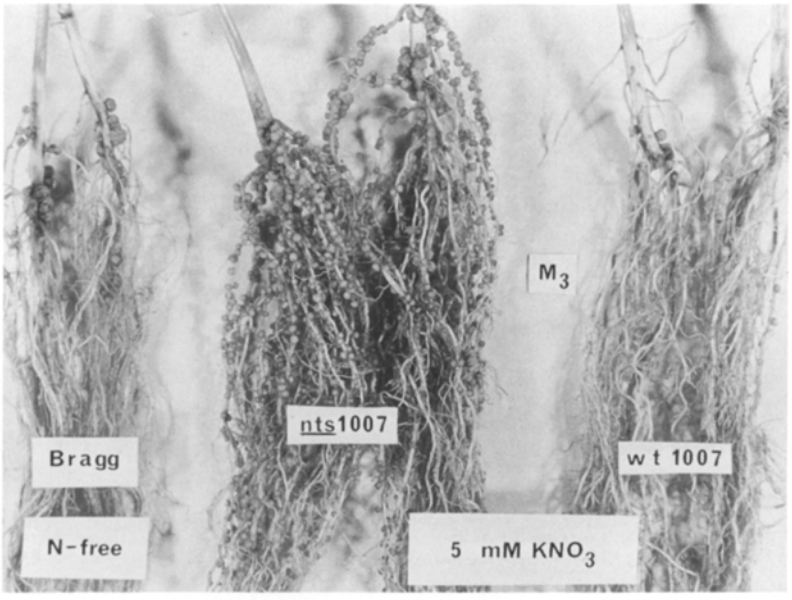

Fig. 1. Nodulation of N_2-dependent Bragg and of nitrate-grown *nts*1007 and wild-type 1007 from a segregating M_3 family. Plants were harvested 49 days after planting. Wild-type Bragg plants received N-free nutrients throughout growth, whereas 1007 M_3 segregants received 5 m*M* KNO_3. All plants were inoculated at day 0 and day 4 with *Bradyrhizobium japonicum* strain USDA110. Wild-type plants (*far left and far right*) display the autoregulation response with the early nodules inhibiting subsequent nodule formation; in contrast, the supernodulated plants nodulate profusely on the tap and lateral roots

Table 3. Estimation of the GECN from the frequency of homozygous mutants within M_2 families. The mean GECN over the 8 families was 1.8 ± 0.3 ($\pm$ SE)

M_2 family	Mutant phenotype	Frequency of homozygous mutants in M_2 family[a]	GECN[b]
382	supernodulating	2/17	2.1
1007	supernodulating	9/48	1.5
183	supernodulating	3/8	0.7
733	supernodulating	4/38	2.4
2062	supernodulating	2/12	1.5
501	supernodulating	1/6	1.5
772	non-nodulating	2/9	1.1
139	non-nodulating	4/51	3.2

[a] Numerator = no. of homozygous mutant M_2 plants; denominator = total no. of plants in M_2 family
[b] Estimated as described in "Materials and methods"

the homozygous recessive supernodulation phenotype. In other studies, *nod*772 and *nod*139 were shown to be monogenic recessive traits (Mathews 1987). The original M_2 seletions of these non-nodulating mutants were always pure-breeding in the M_3 generations (Carroll et al 1986).

Estimation of the GECN in soybean

Since the recessive mutants described above arose from independent mutated loci, the frequency of the homozygous mutants in their respective M_2 families can be used to estimate the GECN for soybean, as described in "Materials and methods". Thus, the apparent GECN in M_1 seeds 382, 1007, 183, 733, 2062, 501, 772 and 139 are listed in Table 3. The lowest estimate for the GECN, 0.70, was observed in M_2 family 183 whereas the highest estimate came from family 139 (apparent GECN = 3.2). Taking the average for all these M_2 families indicates that the GECN for soybean approximates to two [mean GECN = 1.8 ± 0.3 ($\pm$ SE); Table 3].

Discussion

Soybean is generally considered to be a diploidized tetraploid ($2n = 4x = 40$), based on the chromosome number (x) being equal to ten (Lackey 1981). Despite regular bivalent pairing at meiosis, the degree of meiotic chromosome pairing in haploid soybean ($2n = 20$) is sufficient to indicate polyploid cytogenetic composition and considerable genome duplication in diploid soybean (Crane et al. 1982). It is therefore plausible that some loci are duplicated in this species, but since the non-nodulating (Mathews et al. 1987) and supernodulating mutants discussed in this paper behave as recessive traits, it appears that at least these loci are not duplicated. If this was not so, it would have been impossible to detect recessive variants due to the presence of the wild-type replicate gene. The supernodulating mutants lack autoregulation as typified for *nts*1007 in Fig. 1, and complementation tests between these lines suggests that they are all anomolous in the same gene (Delves et al. 1988). The non-nodulating mutants are divided between two complementation groups, with rj_1, *nod*772 and *nod*49 representing one and *nod*139 representing the other (Mathews 1987). Although other genes in soybean may be pertinent to nodule formation, the detection of recessive variants of these may be masked by the presence of a duplicated locus.

Based on the segregation ratios of M_2 homozygous mutants, the GECN for soybean is approximately two (Table 3). Thus, at the time of mutagenesis there was an average of two germline cells in the seed. The mutagenesis procedure including pre-soaking of seed, ethyl methanesulphonate mutagenesis and post washing took 20–21 h (Carroll et al. 1986), and since the first onset of DNA synthesis in rice, for example, is thought to be at least 24 h after hydration (Sarma et al. 1979), it is probable that replication of the soybean genome did not occur prior to exposure to the mutagen and that two cells (or one cell at the G2 stage of cell division) in the dormant soybean seed are generally the progenitors for the subsequent generation of seeds. This estimate of the GECN in soybean compares, for example, with one for coffee (Moh 1961), two for *Arabidopsis thaliana* L. (Li and Redei 1969) and five or more for corn (Anderson et al. 1949; Johri and Coe 1983). The GECN is of practical significance because it can be used to estimate the rate of recovery of mutants (Redei 1975). The probability of recovering an existing mutant in an M_2 family is dependent on the number of plants screened from this family, the nature of inheritance of the mutation and the GECN. These concepts are illustrated for recessive mutations in Table 4. Obviously, the probability of recovering an existing mutant from an M_2 family increases with the number of plants screened and decreases as the GECN increases (Table 4; also see Redei 1975). In our study, 12 seedlings per M_2 family were usually screened for the supernodulating (*nts*) phenotype. Thus, based on GECN = 2, we recovered almost 80% of the recessive supernodulation mutants present in the M_2 population. On average, 7–8 plants per M_2 family were screened for non-nodulation (Carroll et al. 1986) and 5 were screened for nitrate reductase deficiency in another parallel study (Carroll and Gresshoff 1986). Thus, we probably recovered about 60% and 50%, respectively, of these variants in the

Table 4. The probability of recovering an existing recessive mutant in a M_2 family. Each entry in the table is the probability of finding an existing recessive mutant (P_R) for a specific GECN and a specific no. of plants screened in the M_2 family (n); $P_R = 1-[(4\times GECN-1)/(4\times GECN)]^n$

Plants screened per M_2 family	GECN						
	1	2[a]	3	4	5	6	10
1	0.250	0.125	0.083	0.063	0.050	0.042	0.025
2	0.438	0.234	0.160	0.121	0.098	0.082	0.049
4	0.684	0.414	0.294	0.228	0.185	0.157	0.096
5[b]	0.763	0.487	0.353	0.276	0.226	0.192	0.119
6	0.822	0.551	0.407	0.321	0.265	0.225	0.141
7[c]	0.867	0.607	0.456	0.363	0.302	0.258	0.162
8[c]	0.900	0.656	0.501	0.403	0.337	0.289	0.183
12[d]	0.968	0.799	0.648	0.539	0.460	0.400	0.262

[a] GECN for soybean (Table 3)

[b, c, d] Approximate no. of plants screened per M_2 family for nitrate reductase deficiency (Carroll and Gresshoff 1986), nonnodulation and supernodulation (*nts*), respectively

population, assuming recessive inheritance of nitrate reductase deficiency (Table 4).

The estimate of the GECN for soybean is of value in screening programs for other mutants that may be scientifically or agronomically useful. It is also relevant to mutagenized soybeans that exist as bulk M_2 populations, collectively derived from many M_1 plants. For example, using Table 4 and a GECN of 2, if the number of plants screened in an M_2 population is 12 times the number of M_1 plants that contributed to this population, then it is likely that about 80% of the existing recessive mutants in the population would be recovered. Of course, harvesting M_2 families from individual M_1 plants still allows the advantage of knowing that each mutant selection arose from an independent mutation event.

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