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TESTING OF INDUCED MUTANTS OF WINGED BEAN (*PSOPHOCARPUS TETRAGONOLOBUS* (L.) DC) FOR NODULATION AND PHENOTYPIC PERFORMANCE

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Abstract

Four seed coat colour mutants, which were accompanied with changes in tannin content, were earlier selected from M_3 seeds following gamma radiation of dry seeds of two winged bean (*Psophocarpus tetragonolobus* (L.) DC) cultivars. These mutants were X22 and 3/1-10-2 obtained from cv UPS 122, and 3/4-10-7 and 3/9-0-12 from cv Kade 6/16. They were investigated for nodulation behaviour following inoculation with *Bradyrhizobium*. The two mutants, 3/4-10-7 and 3/9-0-12 produced more nitrogen-fixing nodules per plant than their parent at flowering time. Mutant X22 produced a lower number of nodules than its parent, cv UPS 122, whereas mutant 3/1-10-2 produced the same number. For mutant X22, the peak of nodule production seemed to have been reached already at 45 days after sowing of seeds. Nodulation of the parental cultivars was slower than in the mutants at 45 days after sowing but recovered and was relatively more at flowering time, 76 days days after sowing. Nodule dry weight followed a similar trend with the parents producing a lower amount of nodule tissue than the mutants at 45 days after sowing. Significant differences ($P = 0.05$) were recorded for the number of nodules per plant but not for the nodule dry weight. Earlier nodulation and changes in the number of nodules per plant observed in the mutants can be attributed to mutations in the flavonoid biosynthetic pathway that also influenced seed-coat colour. The desired mutant,3/4-10-7 with a low tannin content, clearly showed an improved nodulation.

Introduction

Majority of leguminosae are characterized by development of root nodules in symbiotic relationship with soil bacteria of the genera, *Rhizobium, Bradyrhizobium* and/or *Azorhizobium*. These bacteria can infest the roots of a specified host plant and induce the formation of nodules which are developed from newly-formed meristems in the root cortex. It is within these specialized organs, called nodules, that the bacteria inhabit for fixation of atmospheric nitrogen. The rhizobial nodulation genes required for the induction of the nodulation process, the *nod* genes and the plant genes that are induced during the nodule formation, the *nodulin* genes, have partly been identified (Fisher & Long, 1992; Schlaman, Okker & Lugtenberg, 1992; Long & Staskewics, 1993). The *nod* genes are in turn induced by flavonoids, which are a group of aromatic rings held together by a C3 unit.

Synthesis of the flavonoids in the presence of the enzyme chalcone synthase 4 (CHS), starts with the condensation of one molecule of 4-coumaroyl-CoA and three molecules of malonyl-CoA to produce naringenin chalcone. Isomerization of the naringenin chalcone by chalcone flavonone isomerase (CHI) yields naringenin flavonone. In addition to these *nod* gene inducing flavonoids, there are other flavonoids that inhibit *nod* gene expression (Firmin *et al.*, 1986; Djordjevic *et al.*, 1987; Long, 1989); for example, the isoflavone daidzein induces *nod* gene expression in *Bradyrhizobium japonicum* but it is an inhibitor

in *Rhizobium trifolii* and *R. leguminosarum* (Quattrocchio, 1994). The composition of the mixture of flavonoids in exudates released by roots varies between legumes (Peters & Long, 1988) and, therefore, the induction step by the flavonoids may determine host plant specificity of nodulation (Recourt, 1991).

Winged bean (*Psophocarpus tetragonolobus* (L.) DC) has been described as the best nodulating legume (Anon., 1981). This crop effectively forms a symbiotic relationship with a wide range of bacteria within the *Bradyrhizobium* spp. (Ikram & Broughton, 1980; Broughton *et al.*, 1984). Masefield (1957) recorded that winged bean produced greater numbers as well as higher weight of nodules than other legumes including *Vigna unguiculata, Phaseolus vulgaris, Arachis hypogaea, Glycine max, Pisum sativum, Phaseolus aureus, Pachyrhizus erosus, Canovalis gladiata, Dolichos lablab and Vigna subterranea.* Harding, Lugo Lopez & Pariz-Escobar (1978) have also reported that greater numbers of nodules and heavier dry weights of nodules have been found on winged bean roots than on other legumes when inoculated or grown in soils with no previous record of legume cultivation.

This not withstanding, there have been seemingly contrasting reports on winged bean nodulation. Whereas Masefield (1973) noted that good nodulation was obtained wherever the crop had been grown irrespective of inoculation of the seeds, Rachie & Roberts (1974) have reported poor nodulation or lack of efficient rhizobia in parts of Nigeria. These differences have been attributed to the observation that different legume genotypes respond differently to nodulation and nitrogen fixation (Caldwell & West, 1977; Herath, Dharmawanza & Omrodd, 1978; Iruthayathas & Herath, 1981; Nutman, 1984).

Natural populations and induced mutations have provided genetic variation in host plants for an altered symbiotic interaction (Postma *et al.*, 1988). The use of induced mutations in this regard has been documented. For example, a supernodulating mutant (Jacobsen & Feenstra, 1984) and nodulation-resistant mutants (Jacobsen, 1984) have been selected after mutagenic treatment of seeds of *Pisum sativum* cv Rondo with ethyl methyl sulphonate. Supernodulating mutants of *Glycine max* have also been documented (Carrol *et al.*, 1985). The symbiotic behaviour of the host plant can be modified by induced mutations (Jacobsen, 1984). Mutant lines with different tannin content of seeds have earlier been selected in a winged bean mutation breeding programme (Klu *et al.* 1997). Tannins are end products of the above-mentioned flavonoid biosynthetic pathway; therefore, it is worthwhile to test them for their nodulation ability. The objective of this study, therefore, was to examine the nodulation and other phenotypic characters in some winged bean seed-coat-colour mutant lines with respect to the tannin content of the seeds.

Experimental

Plant material

Seeds of winged bean cultivars UPS 122 and Kade 6/16 and the M_4 seeds of seed coat colour mutants, 3/1-10-2 and X22 from cv UPS 122 and 3/4-10-7 and 3/9-0-12 from cv Kade 6/16 were used. The colour changed from black in UPS 122 to shades of brown in the mutants 3/1-10-2 and X22; and from brown to light brown in mutant 3/4-10-7 and light brown with a saddle-shaped region around the hilum in mutant 3/9-0-12. The parents were cv Kade 6/16 and cv UPS 122. Cv Kade 6/16 was white flowering with green seedlings and cv UPS 122 was pink flowering with purple seedlings. The tannin content of the whole seeds of cv UPS 122 was 1.7 mg CE /g and that of cv Kade 6/16 was 1.36 mg CE/ g. The mutants have also been tested for tannin content in their seeds. The mutant 3/4-10-7 had a reduced tannin content of 0.37 mg CE / g seed sample. The other mutants had higher tannin contents than their parents, varying from 1.96 to 2.50 mg CE/g [25].

Experimental conditions

All the nodulation experiments were set up in a plastic house in which the mean day and night

temperatures were 35 °C and 23 °C respectively. Plants were sown in Rhondic Nitisol soil (local name, Adenta series) with *p*H (1:1 soil:water) being 5.0, a total nitrogen content of 0.04 per cent and available phosphorus (Bray 1) of 5.5 ppm was used. The soil was air dried and sieved through a 5 mm mesh sieve. Plastic pots (with holes at the bottom) which have a height of 16.0 cm, a width of 18.0 cm at the top and 11.0 cm at the base were each field with 3 kg soil and were watered regularly. A mixture of two local *Brahyrhizobium* isolates, (labelled LWB3 and LWB8) obtained from the winged bean cultivars UPS 122 and Kade 6/16 growing in the field were used. Each isolate was grown separately in yeast extract mannitol (YEM) broth for 7 days to a cell density of about 10^9 ml^{-1} on a rotary shaker operating at 1000 rpm.

Nodulation of seedlings

Each pot was seeded with four seeds of a winged bean line listed earlier. Each pot of soil was innoculated with 3 ml. of a 1:1 ratio mixture of the *Bradyrhizobium* isolates just after sowing of the seeds. Seedlings were thinned to one, a week after emergence. Plants were watered daily with tap water and once a week with 50 ml Hoagland nutrient solution (Hoagland & Aron, 1938). Each winged bean line was replicated four times and pots were arranged in a randomized complete block design on raised benches. Plants were harvested 45 days after sowing of seeds. In another experiment, plants were harvested 76 days after emergence which was when all plants were flow-

TABLE 1

*Number of nodules, nodule dry weight and shoot dry weight per plant for four winged bean mutants and their parents**

	Mean values at 45 days after sowing of seeds		*Mean values at 76 days (flowering time)*		
Winged bean line	*Nodule No. per plant*	*Nodule dry weight (g) per plant*	*Nodule No. per plant*	*Nodule dry weight (g) per plant*	*Shoot dry weight (g) per plant*
UPS 122 (Parent)	12.00bc	0.09d	35.50xy	0.70e	6.93f
3/1-10-2 (Mutant)	20.60ab	0.21d	36.00xy	0.81e	6.08f
X22 (Mutant)	21.80ab	0.28d	22.80yz	0.68e	6.22f
Kade 6/16 (Parent)	6.90c	0.14d	18.00z	0.50e	7.53f
3/4-10-7 (Mutant)	21.40ab	0.24d	29.00xyz	0.60e	7.58f
3/9-01-2 (Mutant)	30.90a	0.26d	42.00x	0.72e	8.01f

Means with the same letter in the same column are not significanttly different from each other (P=0.05) by Duncan's multiple range test.

ering. Roots were washed free of soil and the nodules, removed, counted and dried at 65 °C and weighed.

Phenotypic performance of the mutants

The mutants and their parents were also sown in the field at 1 m × 1 m spacing with 2 m interplot spacing to raise an M_4 population. The seeds were sown in April which was the time for the onset of the major raining season. The plants were supported on 2-m wooden stakes and records were taken on flowering time, maturity time, the lengths of dry pods harvested from five plants randomly selected on each plot, number of seeds per pod harvested from a plant and weights of sets of 100 seeds per 10 plants randomly selected on a plot.

Results

Nodulation

Records on nodulation of the winged bean cultivars UPS 122 and Kade 3/9-0-12 and their mutants are presented in Table 1. The parental cultivars, UPS 122 and Kade 6/16 produced mean nodule numbers of 12.01 and 6.91 per plant, respectively, at 45 days after sowing of seeds. These numbers were increased about three fold at 76 days after sowing to 35.51 and 18.07 nodules for cvs UPS 122 and Kade 6/16. There was a significant difference in nodulation among the cultivars at maturity with the pink flowering cv UPS 122 being the better nodulator (Table 1). In comparison with 45 days after sowing, there were seven and five fold increase in the nodule dry weight at 76 days

TABLE 2

Phenotypic data on the performance of M_4 lines of winged bean mutants and their parents

Winged bean line	*Flowering time*		*Maturity time*		*Pod length (cm)*	*No. of seeds per pod*	*100 seed weight (g)*	*Tannin content of whole seed (mg *CE/g sample)*
	No. of days for first plant to flower	*No. of days for 50% of plants to flower*	*No. of days for first pod to mature*	*No. of days for 50% of plants to mature*				
UPS 122 (Parent)	97.9 ±5.9	98.2 ±4.9	108.8 ±6.1	153.2 ±0.8	14.6 ±0.6	12.8 ±0.5	31.6	1.8
X22 (Mutant)	99.5 ±4.9	103.6 ±5.8	115.0 ±4.8	158.2 ±0.8	13.4 ± 0.8	11.8 ±0.2	29.5	2.5
3/1-10-2 (Mutant)	101.3 ±1.5	109.2 ±1.5	112.4 ±3.4	159.4 ± 0.9	14.9 ±0.8	10.9 ±0.2	29.9	2.0
Kade 6/16 (Parent)	98.2 ±6.4	102.8 ±5.2	112.6 ±7.2	155.2 ±0.9	14.4 ±0.8	14.9 ±0.5	28.6	1.4
3/4-10-7 (Mutant)	102.2 ±3.4	105.2 ±3.4	110.5 ±4.2	160.5 ±0.7	14.5 ±0.4	13.4 ±0.1	28.5	0.4
3/9-0-12 (Mutant)	101.2 ±3.3	103.2 ±4.9	116.0 ±4.3	159.2 ±0.8	14.5 ±0.8	12.2 ±1.0	34.6	2.2

*CE/g - Catechin equivalent per gram

but there was no significant difference among the cultivars.

The mutants, 3/1-10-2 and X22, which originated from cv UPS 122 produced more nodules per plant than their parent at 45 days after sowing. However, after 76 days the situation changed. These two mutants differed both from each other and from their parent in one way or the other. The nodule number per plant for mutant 3/1-10-2 increased about 1.75 times to 36.0, a final number of nodules that was not significantly different from the value of the parent (Table 1). The mutant, X22, on the other hand, seemed to reach its peak of nodulation earlier at 45 days. The two mutants are, therefore, quicker nodulators than their parent but X22 had the lowest number of nodules per plant at 76 days. However, the dry weights of the nodules at this stage of plant growth did not clearly differ between the mutants and their parent.

The observations among the mutants 3/4-10-7 and 3/9-0-12 and their parent cv Kade 6/16 followed a similar trend. After 45 and 76 days of sowing of seeds, the number of nodules per plant for the mutants was higher than those recorded for the parent (Table 1). Increases in nodule number between 45 and 76 days were about 1.5 times to 29.01 and 42.01 for 3/4-10-7 and 3/9-0-12, respectively, while it was 3 times for the parent. These mutants appeared also to be quicker and heavier nodulators than their parent cv Kade 6/16 (Table 1). The desired mutant, 3/4-10-7, with a low tannin content, showed in comparison with cv Kade 6/16 an improved nodulation.

Shoot dry weight

Seedlings of the parental cultivars were at the beginning relatively more vigorous than their mutants. However, at 45 and 76 days after sowing of seeds no major phenotypic differences were observed among the plants of all genotypes. Records on shoot dry weight at 76 days after sowing are presented in Table 1. There were no statistical differences in this trait among the mutants and their parents; although cv Kade 6/16 and its mutants seemed to have higher shoot dry weight than cv UPS 122 and its mutants (Table 1).

Phenotypic performance

Phenotypic data on winged bean cvs UPS 122 and Kade 6/16 and on M_4 lines of their mutants are presented in Table 2. The number of days to the opening of the first flower seemed to be about the same for both parent cultivars. Although there seemed to be no difference in this trait among the winged bean parental lines, all mutants seemed to flower a little later. Pod maturity in the mutants X22 and 3/1-10-2 followed the same trend as observed for flowering. However, pods of the mutant 3/4-10-7, which had the lowest level of tannin, matured about 2-6 days earlier than its parent cultivar Kade 6/16 and the other mutant 3/9-0-12 (Table 2). Pod length and the number of seeds per pod did not seem to differ in the winged bean lines. The heaviest seeds were recorded for the mutant 3/9-0-12. The mutant 3/4-10-7, although it had about the same seed weight as its parent, had a lower tannin content. Seeds of cv UPS 122 were heavier than those of its mutants, 3/1-10-2 and X22.

Discussion

Variability in nodulation among different winged bean accessions has been documented by Iruthayathas & Herath (1981) and Iruthayathas & Vlassak (1982). It has also been recorded that nodulation in this crop commences 2 weeks after sowing of seeds and that after 3 weeks nodules begin to reach their bacteriod stage and by 4 weeks after sowing of seeds, considerable numbers of fully-developed nodules are formed (Iruthayathas & Herath, 1981). The nodulation studies reported here were carried out 45 days after sowing and at flowering time. These are, accordingly, appropriate times for examination of nodulation potentials of the winged bean cultivars and their mutants. The parents had significant differences with respect to the number of nodules formed per plant. The comparison with the mutants clearly indicates that whereas the parent winged bean cultivars, UPS 122 and Kade 6/16 seem to be slower in nod-

ule development, their number increased gradually with time indicating that the mutants were early nodulators.

In cv UPS 122, the increase from 12.00 nodules per plant at 45 days to 35.50 nodules per plant at flowering time exceeded increases from 20.61 and 21.81 to 36.01 and 22.81 nodules per plant, respectively, for the mutants 3/1-10-2 and X22. Similarly, a three-fold increase from 6.91 to 18.01 nodules per plant for cv Kade 6/16 was more than the increases from 21.41 and 30.91 to 29.01 and 42.01 nodules per plant, respectively, for the mutants 3/4-10-7 and 3/9-0-12 (Table 1). The differences in nodulation between the parental lines was partly due to the observation within different legume species that genotypes can respond differently to nodulation and nitrogen fixation (Caldwell & West, 1977; Herath, Dharmawanza & Omrodd, 1978, Iruthayathas, Vlassak & Laeremans, 1984).

The phenomenon has earlier been documented in 80 cultivars of soybean (*Glycine max*) which have exhibited considerable variation in nodulation (Graham & Temple, 1984; Okereke & Unaegbu, 1992); and also in bean (*Phaseolus vulgaris*) in which over 600 cultivars were examined for this phenomenon (Graham & Rosas, 1977; Graham, 1981; Graham & Temple, 1984). Flavonoids are involved in the induction of *nod* genes for nodulation (Chappel & Hahlbrock, 1984; Peters, Frost & Long, 1986; Remond *et al.*, 1986; Zaat *et al.*, 1987). However, there are also certain flavonoids which inhibit bacterial *nod* gene action (Firmin *et al.*, 1986; Djordjevic *et al.*, 1987; Peters & Long, 1988; Long, 1989). Mutations in the flavonoid biosynthetic pathway could automatically affect the actions of the inducers and/or inhibitors, leading to changes in the signals of the root exudate of the host plant to the rhizobial bacteria (Recourt, 1991).

Accumulation of naringenin is highly reversible and not inhibited by the presence of other flavonoids (Recourt, 1991). However, mutations in the structure of the naringenin flavone could offset such a system, and, ultimately, *nod* gene activity. Mutations could also effect the enzymatic steps in the biosynthetic pathway. The changes in nodulation, as described for the mutants, could be attributed to the possible mutations outlined. since mutations alterting nodulation may be root and/or shoot factor specific (Delves *et al.*, 1986). Host plant genes are directly or indirectly involved in nodule formation and nodule functioning (Postma, 1990). The successful use of induced mutations to create variability in the host plant for differences in nodulation has been described in several legumes. These include *Pisum sativum* (Jacobsen, 1984; Jacobsen & Feenstra, 1984), *Vigna radiata* (Micke, 1984) and *Glycine max* (Carrol *et al.*, 1985).

It appears the variability created for nodulation by mutations in the investigations is the first reported one in winged bean. It is remarkable that in this experiment, all the selected mutants with altered seed coat colour showed this pleiotropic effect. There are not many examples described in the literature in which mutants with an altered seed coat colour and tannin content were systematically investigated for their nodulation behaviour. The results have clearly indicated that selection for seed coat colour changes can be used as means to select for altered symbiotic performance. The mutated host plant genes involved in this process in the winged bean need to be investigated. Colour mutants are an indirect way for obtaining plants with an altered nodulation in which the flavonoid biosynthetic pathway is particularly involved.

Although the winged bean mutants were slow growers as compared to their parents at the seedling stage (data not shown), no dramatic phenotypic differences were observed in the mature plants. The mutants seemed to flower and mature later than their parents. On the other hand, the mutant 3/9-0-12, which had a lower number of seeds per pod than the parent, had heavier seeds and an increase in tannin content. The major effect recorded among the mutants is that the mutant 3/4-10-7, which had a reduced tannin content, had an increased nodule number as compared to the parent.

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