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Comparison of two *Pisum sativum* nodulation mutants with their parental cultivar

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Abstract

In comparison with the parental cv. Finale the 'RisfixC' supernodulator exhibited higher, continuously increasing nodule number and fresh mass accumulation, but substantially lower individual nodule fresh mass, leghemoglobin concentration, and specific acetylene reduction activity of nodule tissue. There were no substantial differences between Finale and 'RisfixC' in total acetylene reduction, nodule leghemoglobin accumulation per nodulated root, total and specific CO_2 evolution from nodulated roots and gross CO_2 respiratory costs of acetylene reduction. The 'RisfixC' also exhibited a substantially lower plant dry mass production (by 30 %), but nitrogen concentration in shoots and carotenoid concentration in leaf tissue were significantly higher by 33 and 14 %, and the chlorophyll a+b content insignificantly higher than in the parental cultivar. In contrast, the nodulation mutant 'Risnod29', exhibited a somewhat higher nodule fresh mass accumulation (by 21 %) and individual nodule fresh mass (by 23 %), total and specific acetylene reduction (by 49 and 19 %) and a somewhat more rapid plant dry mass accumulation compared with the cv. Finale.

Introduction

Chemical mutagenesis has given rise to a number of symbiotic mutants, *e.g.*, in soybeans (Carroll *et al.* 1984, Carroll *et al.* 1985a,b, Gremaud and Harper 1989, Akao and Kouchi 1992), peas (Jacobsen 1984, Jacobsen and Feenstra 1984, Kneen and LaRue 1984) and common beans (Davies *et al.* 1988, Park and Buttery 1988, 1990, 1992). Recently, Engvild (1987) published a large set of nodulation mutants of *Pisum sativum* L. Based on the phenotypic classification of these mutants (Novák *et al.* 1993a), among others, new nitrate-tolerant supernodulator 'RisfixC' (Novák *et al.* 1993b) and a Nod+Fix+ 'Risnod29' mutant were identified. In contrast to the original classification of the 'RisfixC' as Nod+Fix- and 'Risnod29' as Nod- (Engvild 1987) we found these mutants to be nitrate-tolerant Nod+++Fix+ ('RisfixC') and Nod+Fix+

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('Risnod29'), respectively. The 'Risnod29' exhibited somewhat higher nodule dry mass accumulation, single nodule fresh mass, and dry mass and N accumulation in shoots relative to its wild-type parental cv. Finale (Novák *et al.* 1993a).

The objective of the present study is a more detailed characterization of the basic symbiotic, growth and some physiological traits in both the mutants and their parental cv. Finale as influenced by plant ontogeny.

Materials and methods

Garden pea (*Pisum sativum* L. cv. Finale, wild-type, Nod+Fix+) and its nitrate-tolerant supernodulator (nts, Nod+++Fix+) 'RisfixC' and a nodulation 'Risnod29' (Nod+Fix+) mutant (Novák *et al.* 1993a) were used. The cv. Finale and 'RisfixC' and 'Risnod29' mutants, originally classified as Nod+Fix- and Nod-, respectively (Engvild 1987), were obtained from Dr. K.C. Engvild, Risø National Laboratory, Roskilde, Denmark. Test plants were grown in plastic tubes containing perlite saturated with a nutrient solution (Škrdleta *et al.* 1980) with 76 mg dm^{-3} $Ca(NO_3)_2.H_2O$ - a nodulation and dinitrogen fixation supporting nitrate level (Škrdleta *et al.* 1984). The tube bottoms were closed with cotton mull allowing roots to grow through. The nutrient solution was exchanged twice a week. Two-day-old seedlings were inoculated with 3 cm^3 of inoculum of *Rhizobium leguminosarum* bv. *viceae* strain 248 at a rate of 7 × 10^7 viable bacteria per seedling. The peas were grown under environmentally-controlled conditions (16/8 h day/night photoperiod, 21/14 °C air teperature, 77/87 % RH and irradiance at a plant top level of about 500 μmol m^{-2} s^{-1}) in the *S10H Conviron* (Winnipeg, Canada) growth chamber (Škrdleta *et al.* 1991). 5 h after the light was switch on, plants were randomly sampled (n = 6) and their intact nodulated roots were immediately incubated individually in 100-cm^3 serum bottles at 23 °C for 30 min. 1 cm^3 samples of the incubation atmosphere were withdrawn after incubation to estimate CO_2 evolution from nodulated roots. The incubation vessels were aerated for 1 min and, after addition of C_2H_2 (partial pressure of C_2H_2 *ca.* 10 kPa), incubated for further 33 min to determine C_2H_4 production at 3 and 33 min of incubation (AR). Concentrations of CO_2, C_2H_2, and C_2H_4 in 0.5-cm^3 subsamples were determined by gas chromatography. Regardless of potential errors associated with C_2H_2 reduction in closed system (Minchin *et al.* 1986) and a smaller acetylene-induced decline in C_2H_2-reducing activity exhibited by some supernodulators (Schuller *et al.* 1988, Rosendahl *et al.* 1989), this procedure is believed to yield a reliable data on acetylene reduction for purposes of comparison among the genotypes and plant growth stages. Leghemoglobin content was determined according to Wilson and Reisenauer (1963) in nodules stored at -20 °C before assay. Chlorophyll *a*+*b* and carotenoid contents in leaf tissue (Wettstein 1957) were determined in 0.33 cm^2 leaf discs from the topmost, fully expanded leaf. The leaf discs were also stored at -20 °C before assay. The dry plant shoots were combined from two plants of the respective treatment, fine-ground and analyzed for nitrogen content (n = 3) by automated Dumas method (*CHN analyzer, Leco Co.*, Saint Joseph, USA).

Results and discussion

In relation to the parental cv. Finale, the stems of 'Risnod29' 34-, 41- and 48-d-old plants (from early flowering to early pod-filling stage), were considerably longer (by 39, 26 and 11 %, respectively). On the contrary, stem of 'RisfixC' was shorter by *ca.* 11 % (a season average; not documented).

Table 1. Changes in TAR, SAR, leghemoglobin accumulation, leaf chlorophyll *a*+*b* content and plant dry mass accumulation during growth of the supernodulator 'RisfixC', symbiotic mutant 'Risnod29' and their parental cv. Finale.*

Genotypes	Plant age [d]				
	27	34	41	48	55
	TAR [nmol C_2H_4 root^{-1} s^{-1}]				
'RisfixC	3.6 a,b	ND	2.4 b,c	2.3 b,c	0.6 d
'Risnod29'	2.8 a-c	ND	2.6 b,c	4.2 a	2.7 b,c
cv. Finale	3.1 a-c	ND	1.6 c,d	1.9 c,d	2.7 b,c
	SAR [nmol C_2H_4 g^{-1} (nodule FM) s^{-1}]				
'RisfixC	2.2 d	ND	1.0 e	0.9 e	0.2 e
'Risnod29'	5.0 a	ND	3.3 b,c	3.8 b	2.9 c,d
cv. Finale	5.0 a	ND	2.8 c,d	2.6 c,d	2.7 c,d
	Leghemoglobin accumulation [mg (nodulated root)$^{-1}$]				
'RisfixC	4.14b-e	4.13b-e	3.64c-e	6.22a	4.91a-c
'Risnod29'	3.05d,e	4.02b-e	3.94c-e	5.66a,b	4.85a-e
cv. Finale	3.20c-e	3.37c-e	2.90e	3.54c-e	4.77a-d
	chlorophyll *a*+*b* content [µg cm^{-2}]				
'RisfixC	64.2 a	59.3 a,b	58.8 a,b	62.9 a	44.5 c
'Risnod29'	61.7 a,b	59.9 a,b	52.6 b	53.2 b	36.2 d
cv. Finale	56.8 a,b	56.2 a,b	57.3 a,b	60.4 a,b	40.1 c,d
	dry mass accumulation [g plant^{-1}]				
'RisfixC	0.56b	0.96b	1.73b	3.32b	4.78b
'Risnod29'	0.79a,b	1.74a	2.98a	6.05a	6.14a,b
cv. Finale	0.98a	1.62a	2.23b	4.21a,b	6.49a

*Values within the rows and columns followed by the same letter are not significantly different at $P \leq 0.05$ on the basis of Duncan's multiple range test; ND - not determined.

As expected, the whole plant dry mass accumulation in 'RisfixC' supernodulator was consistently lower approximately by 39 % in relation to Finale and 'Risnod29' (Table 1). In this respect, apparently, 'RisfixC' had a restricted growth as also exhibited, *e.g.*, by soybean (Carroll *et al.* 1985a, Day *et al.* 1986, Hansen *et al.* 1989, Lee *et al.* 1991, Francisco *et al.* 1992) and pea supernodulators (Rosendahl *et al.* 1989). However, in the common bean supernodulator 'R32' plant growth was similar to, or somewhat less than that of the parent (Park and Buttery 1990).

Nitrogen concentrations in shoot dry mass were mostly similar in cv. Finale and

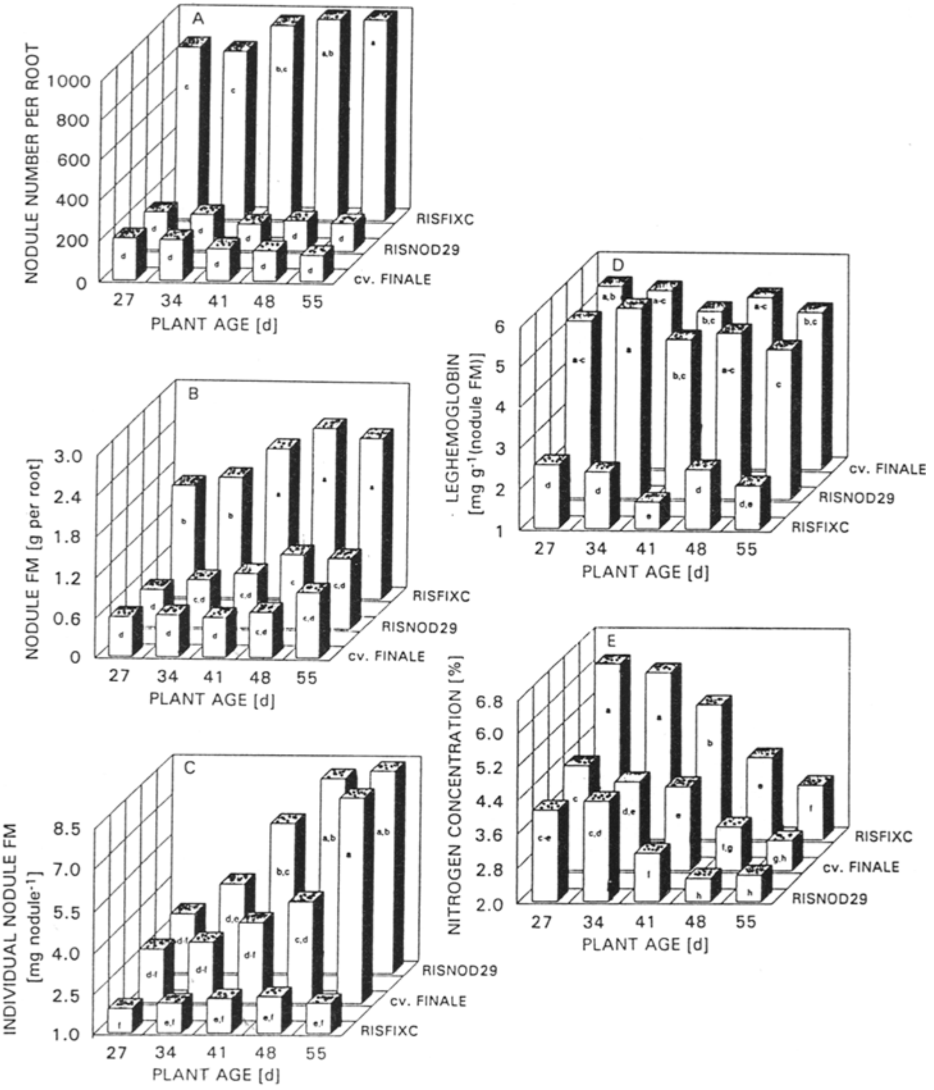

Fig. 1. Changes in nodule number (*A*), nodule fresh mass (FM) accumulation (*B*), individual nodule fresh mass (*C*), leghemoglobin concentration in nodule tissue (*D*), and nitrogen concentration percentage in shoot dry mass (E) during plant growth for the supernodulator 'RisfixC', symbiotic mutant 'Risnod29' and their parental cv. Finale. Values presented as blocks marked with the same letter are not significantly different at $P \leq 0.05$ on the basis of Duncan's multiple range test.

'Risnod29' and during ontogeny. However, values for the 'RisfixC' were constantly higher and decreased from 6.2 to 3.3 % during plant growth (Fig. 1E). Chlorophyll *a*+*b* content of the leaf tissue in the supernodulator tended to be higher compared with cv. Finale. 40-d-old 'RisfixC' plants had a significantly higher leaf tissue carotenoid concentration (8.8 μg cm^{-2}) than cv. Finale and 'Risnod29' (7.7 and 7.3 μg cm^{-2}). A lower plant dry mass accumulation (Table 1) but a higher nitrogen concentration of the 'RisfixC' shoot dry mass (Fig. 1E) resulted in a similar nitrogen content (144, 151 and 164 mg plant^{-1} for the 55-d-old 'Risnod29', 'RisfixC' and cv. Finale plants, respectively).

In comparison with the parental cv. Finale and 'Risnod29' the 'RisfixC' exhibited a much higher, continuously increasing number of nodules per root (Fig. 1A), nodule fresh mass accumulation (Fig. 1B) and lower individual nodule fresh mass (Fig. 1C). In the 34- to 41-d-old 'RisfixC', the nodule biomass constituted as much as 45 % of the nodulated root system, whereas in cv. Finale and 'Risnod29' nodules only represented *ca.* 10 and 14 % (Table 2).

Table 2. Total and specific CO_2 efflux from nodulated roots, gross CO_2 respiratory costs of acetylene reduction and nodule/nodulated root FM ratio in the supernodulator 'RisfixC', mutant 'Risnod29', and their parental cv. Finale.

Plant age [d]	Genotypes	Total CO_2 efflux [nmol root^{-1} s^{-1}]	Specific CO_2 efflux [nmol g^{-1}(FM) s^{-1}]	CO_2 costs of C_2H_2 reduction [nmol nmol^{-1}]	Nodule/nodulated root FM ratio [%]
34	'RisfixC'	6.3 a	1.6 a	7.4 a	45.3 a
	'Risnod29'	6.9 a	1.1 b	5.2 a	11.6 b
	cv. Finale	7.2 a	1.2 b	6.6 a	10.4 b
41	'RisfixC'	8.1 a	1.6 a	4.2 a	43.9 a
	'Risnod29'	9.1 a	1.8 a	3.7 a	16.0 b
	cv. Finale	8.0 a	1.4 a	5.3 a	10.4 c

Values followed by the same letter are not significantly different at $P \leq 0.05$ on the basis of Duncan's multiple range test.

The 'RisfixC' root nodules were not only smaller (Fig. 1C) but also had a much lower leghemoglobin concentration (Fig. 1D) and their specific AR (SAR, Table 1) was much lower. Day *et al.* (1987) and Hansen *et al.* (1990) observed the same relative differences in their *nts*382 soybean supernodulator in relation to the parental cv. Bragg and Day *et al.* (1987) found these lower values to be directly related to a lower nodule bacteroid content. A lower soluble protein concentration in the nodule tissue of the pea *nod3* supernodulator, supporting a lower bacteroid content, was also documented by Rosendahl *et al.* (1989) and a lower SAR by Jacobsen and Feenstra (1984). While SAR and specific leghemoglobin concentration were lower in 'RisfixC' than in cv. Finale and 'Risnod29', total AR (TAR) and nodule leghemoglobin accumulation, when expressed per whole nodulated root, were very similar (Table 1).

In agreement with these findings almost insignificant differences were found in total and specific CO_2 evolution from nodulated roots and, reasonably, in gross CO_2 respiratory costs of acetylene reduction (Table 2). It does not, however, rule out the possibility that specific respiratory CO_2 efflux for growth and maintenance of the nodulated root system might be higher in a supernodulator (Rosendahl *et al.* 1989).

A great reduction in plant biomass, individual nodule mass, leghemoglobin concentration, SAR, and a high N concentration in shoot dry mass, as documented in our nitrate-tolerant 'RisfixC', are considered (Hansen *et al.* 1990) to be the consequence of a limited photosynthetic potential and/or a carbon limitation in some legume supernodulators. However, despite differences in the specific nodule activities and plant biomass accumulation, the nitrogen content in shoot dry mass, TAR and leghemoglobin accumulation per nodulated root were suprisingly similar across the genotypes indicating the presence of a common regulatory mechanism in nodule and/or plant N metabolism, as also supposed, *e.g.*, by Francisco *et al.* (1992). Moreover, TAR activities (Table 1) when expressed on the shoot dry mass basis, were higher in the 27- to 48-d-old 'RisfixC' plants in comparison with those of cv. Finale (not documented).

However, as was found, *e.g.*, in the *nts* 1007 (Hansen *et al.* 1992) and *nts* 1116 (Haider *et al.* 1991) soybean supernodulating mutants not all the above mentioned traits are expressed in some supernodulators.

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