

GENETIC VARIATION IN ROOT NODULE FORMATION OF DIFFERENT PEA CULTIVARS

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In a search for a crop plant yielding a high level of seed proteins at a low energy cost, we have analyzed the capacity of some pea cultivars to form nitrogen-fixing root nodules in the presence or absence of nitrogen and various strains of the bacterium Rhizobium leguminosarum. The aim of the project is to elucidate the contribution of the plant and the bacterial genomes in the establishment and function of root nodules efficient in nitrogen fixation.

The six cultivars of pea listed in Table 1 were studied for their capacity to develop root nodules in aerated aqueous medium and in vermiculite moistened with nutrient solution with or without a nitrogen source. The nutrient solution contained per liter of double deionized water: CaHPO<sub>4</sub> 1g; K<sub>2</sub>HPO<sub>4</sub> 0.2g; MgSO<sub>4</sub> 0.2g; NaCl 0.2g; FeCl<sub>2</sub> 0.001g; 1 ml of Hutner trace element solution modified to avoid nitrogen in the medium, and, when required, NH<sub>4</sub>NO<sub>3</sub> 0.5g. During growth, nutrient solution or tap water (a control experiment failed to detect organisms capable of establishing root nodules) was added to the cultures to maintain the salt concentration at about the level given above. The plant cultures were irradiated with 20 (range 9-30) Wm<sup>-2</sup> during a 16h photoperiod and the temperature ranged from 18 to 25C. The seeds were surface sterilized and germinated in sterile nutrient solution. As the roots developed, some of the cultures were inoculated with one of 8 known strains of Rhizobium leguminosarum obtained from the Rothamsted (England) and Uppsala (Sweden) collections. Prior to inoculation the bacterial cultures were washed and resuspended in small volumes of plant nutrient solution deficient in nitrogen. Cultures of the bacterial strains were studied for nitrogen-fixation capacity as measured by hydrogen evolution and activity of uptake-hydrogenase. The strains Roth.V, 312, 313, and 1011 were negative with respect to nitrogen fixation (negative strains), while strain 1008 possessed this activity (positive strain). The time for nodulation, the size, and the number of nodules were recorded. The average number of root nodules per plant is listed in Tables 1 and 2.

It is evident that the pea cultivar 'Monopol', WL 1714 (from the Pisum Gene Bank, Weibullsholm collection) developed more root nodules than the other cultivars studied. The cultivar from Jordan Valley, WL 1293, formed some root nodules when grown on vermiculite (Table 1), but lacked root nodules completely when grown in aqueous culture (Table 2) in the presence or absence of nitrogen (N) and/or Rhizobium. Root nodule formation was completely inhibited by the presence of fixed nitrogen in aqueous cultures (Table 2). However, the presence of nitrogen apparently did not influence the number of root nodules formed by plants developed on vermiculite culture (Table 1). Plants developed in aqueous culture deficient in fixed nitrogen formed roots densely covered with root hairs and some nodules were formed by the cultivars 'Dark Skinned Perfection' and Monopol (WL 1714) even when not inoculated with Rhizobium (Table 2). Inoculation with cultures of Rhizobium leguminosarum resulted in an increased number of root nodules. The cultivar Monopol (WL 1714) developed similar numbers of root nodules when inoculated with a

nitrogen-fixing strain or any of the non-nitrogen-fixing strains tested. The cultivar Dark Skinned Perfection appeared to yield more root nodules when inoculated with the nitrogen-fixing strain.

The cultivar from Jordan Valley, WL 1293, lacking the capacity to form root nodules in aqueous culture even when inoculated with Rhizobium, has been reported (1) and (2) to be deficient in the protein lectin (PEA) in the seed. If lectin is also deficient in the roots of WL 1293, this could be related to the lack of root nodule formation with any of the Rhizobium strains in aqueous cultures.

1. Guldager, P. 1978. Theor. Appl. Genet. 53:241-250.
2. Guldager, P., Bøg-Hansen, T.C. 1979. Protides of Biological Fluids. 27th Fd. 11. Peeters. pp. 401-404.

Table 1. Average number of root nodules developed per pea plant of different cultivars grown on vermiculite with nutrient solution. Rhizobium leguminosarum used for inoculation was strain Roth.V. (The number of plants analyzed for each cultivar is given for each treatment.)

Cultivar or line	Rhizobium present		Rhizobium not added
	N deficient	N added	N added
Fenomen	14	3	11
Dark Skin Perfection	10	10	19
Vinco, WL 1703	19	2	30
Parvus, WL 1107	18	22	1
Monopol, WL 1714	58	72	177
From Jordan Valley WL 1293	18	13	16
Plant per sample	20	20	6

Table 2. Average number of root nodules developed per pea plant of different cultivars grown in aqueous culture and inoculated with different strains of Rhizobium leguminosarum. (The number of plants analyzed for each cultivar is given for each treatment)

Cultivar or line	Average of negative strains	Positive strain 1008 present	N added		
			Rhizobium		None
			Present	Absent	
Dark Skinned Perfection	101; S=54	160	20	0	0
Monopol, WL 1714	150; S=84	150	53	0	0
From Jordan Valley, WL 1293	0	0	0	0	0
Plants per sample	100	6	6-12		

S = standard deviation