

## Use of Supernodulating Mutants in Pea Breeding

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Our genetic studies of symbiotic pea mutants have shown that the *Pisum* genome has multiple genes controlling the following traits: (1) capability of symbiosis with *Rhizobium* nodule bacteria; (2) the number and efficiency of root nodules; (3) nitrogen fixation rate intensity, assessed from nitrogenase activity; (4) duration of active nitrogen fixation; and (5) root biomass accumulation. We conclude that the macrosymbiont plays the key role in the genetic control of nitrogen fixation in nodules and that breeding of legume macrosymbionts for greater efficiency of their symbiosis with rhizobia is promising given that rhizobia are present in any substrate or soil, in the field or in a greenhouse.

Supernodulating mutants deserve special attention of breeders as a means for increasing nodulation and nitrogen fixation. However, these mutants suffer from low productivity. The first attempts to involve supernodulating mutants in breeding were done with soybean, despite being unsuccessful (1). To test the feasibility of utilizing supernodulating mutants in legume breeding as tools for intense nitrogen fixation, we performed large-scale field and greenhouse experiments with pea and investigated the expression of the *nod4* and *nod3* supernodulating genes against various genetic backgrounds (2). The results were used in the development of a breeding program for improving the efficiency of the legume-rhizobium symbiosis (Figure 1).

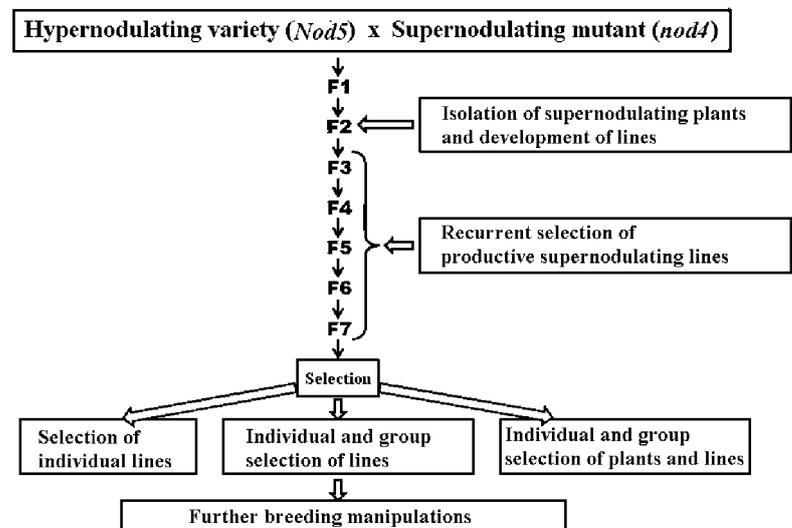


Figure 1. Breeding program for increasing nitrogen fixation in pea *Pisum* s



Figure 2. Pea roots: (1) supernodulating; (2) hypernodulating.

it is important to choose a proper variety, which should be highly productive and hypernodulating. Hypernodulation is characterized by large nodules, forming mainly in the upper and middle portions of roots. The nodules are considerably fewer in number, but larger than in supernodulating forms (Figure 2). Hypernodulation is governed by the dominant allele at the *Nod5* locus located on linkage group (LG) III (3, 4). The pea germplasm collection at Novosibirsk includes pea mutants and varieties with hypernodulation for *Nod5*. The supernodulation in mutant K301, used in our study, has the recessive allele at the *nod4* locus located

Our method of breeding for elevated nitrogen fixation in pea was successfully applied to the development of the pea cultivar Druzhnaya.

**Materials and methods**

Supernodulating plants were selected in F<sub>2</sub>, and individual lines were raised. Productive lines with supernodulation and intense nitrogen fixation were recurrently selected in generations F<sub>3</sub> to F<sub>7</sub>. Performance and nodulation indices in mutant K301, cv. Druzhnaya, and recurrent lines are illustrated in Figure 3.

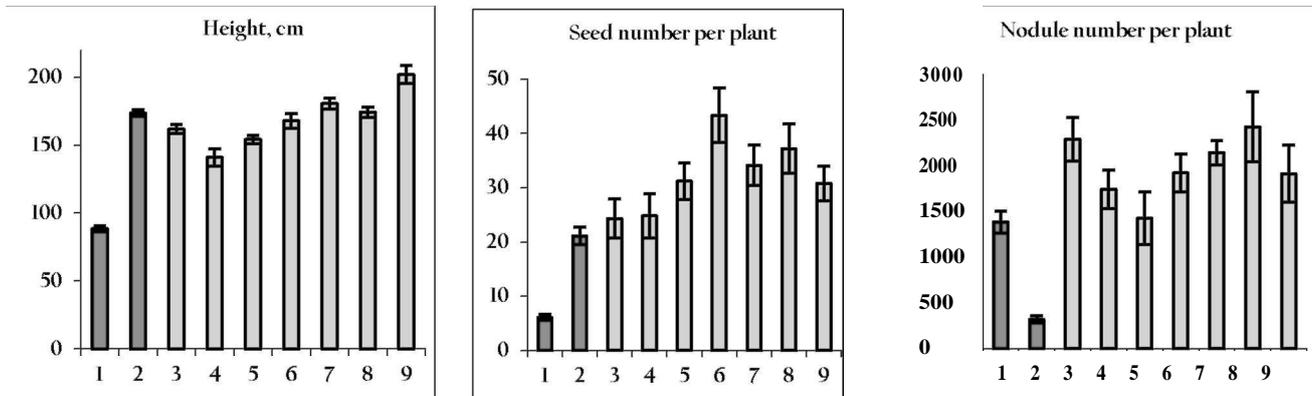


Figure 3. Recurrent F<sub>7</sub> lines obtained from crosses of cv. Druzhnaya to supernodulating mutant K301. Lane 1, K301; lane 2, Druzhnaya; lanes 3-9, recurrent lines.

**Results and discussion**

The supernodulating mutant was both shorter and had less seed production than either Druzhnaya or the recurrent lines. The recurrent lines were slightly taller than Druzhnaya. Many recurrent lines had higher seed yield than Druzhnaya. The recurrent lines considerably outperformed Druzhnaya in symbiosis-related traits, i.e. number of nodules (Figures 3 and 4) and nitrogen fixation rate (Table 1).

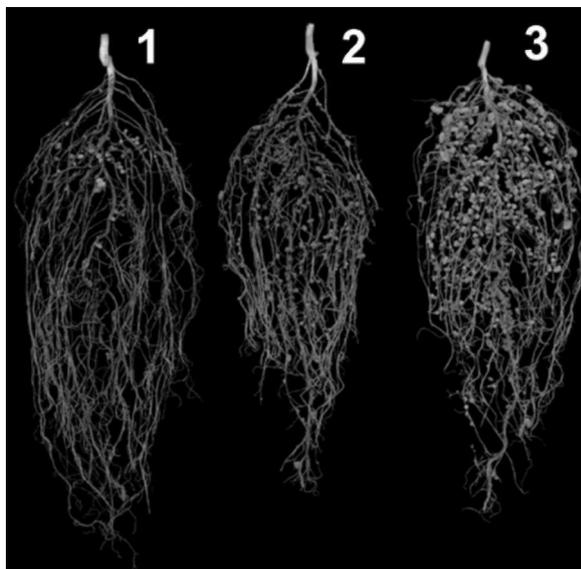


Table 1. Nitrogen fixation in pea cv. Druzhnaya and the recurrent lines.

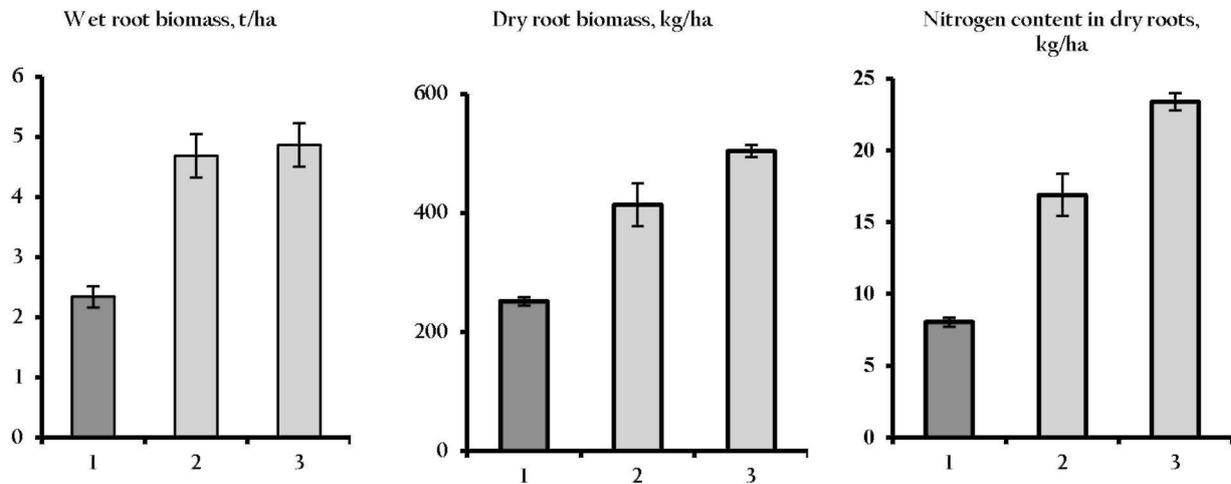
Form	Nitrogenase activity, nmol C <sub>2</sub> H <sub>4</sub> /plant/h
cv. Druzhnaya	424
Supernodulating K301 mutant	1242
Recurrent lines:	
K301 * Druzhnaya	1123-1226
Druzhnaya * K301	2312-2380

Figure 4. Representative examples of pea root biomass of cv. Druzhnaya (Nod5) (1), supernodulating mutant K301 (nod4) (2); and recurrent lines (3).

Individual and group selection of recurrent lines was done in the F<sub>2</sub>. The lines were subjected to further breeding procedures. Similar results were obtained with cv. Novosibirskaya, also crossed to K301.

### Conclusion

Legumes are good rotation crops. We have shown that broad expansion of the pea root system is possible based on results from the recurrent lines. All recurrent lines significantly surpassed cv. Druzhnaya in vegetative root biomass and nitrogen content (Figure 5).



The calculations were performed by the conventional method: 1,800,000 plants per hectare.

**Figure 5.** Accumulation of crude root biomass and nitrogen contents in pea forms: 1 cv. Druzhnaya; 2, 3, recurrent lines.

Our results indicate that forms approaching commercial varieties in performance and symbiosis parameters and even outperforming them in the accumulation of root biomass can be obtained by combining dominant and recessive alleles of different symbiosis-related genes. The cultivation of such forms will improve soil fertility and save mineral fertilizers, which is important with respect to environment protection and energy saving.

### References

1. Bhatia C.R., Nichterlein K., and Maluszynski M. 2001 Euphytica, 120: 415-432.
2. Sidorova K.K., Shumny V.K., and Nazaryuk V.M. 2006 Symbiotic nitrogen fixation: genetic, breeding, ecological, and agrochemical aspects, Geo, Novosibirsk, P. 134 (in Russian).
3. Sidorova K.K. and Shumny V.K. 1997 Doklady Akademii Nauk 353(5):703-704. (in Russian).
4. Sidorova K.K., Shumny V.K., and Mishchenko, T.M. 1999 Doklady Akademii Nauk 367(6):851-852 (in Russian).
5. Sidorova K.K. and Uzhintseva L.P. 1994 Doklady Akademii Nauk, 1994 336(6):847-849 (in Russian).