## The *neoplastic pod* gene (Np) may be a factor for resistance to the pest *Bruchus pisorum* L.

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The dominant gene Np (neoplastic pod) of garden pea (Pisum sativum L.), is known to cause the proliferation of epidermal cells outside the pods bringing about pustular-like formations under reduced illumination, especially in a greenhouse (1, 2). The neoplastic phenotype looks like a true disease which has no advantage to a plant. Nevertheless, the gene turned out to be common in numerous pea forms, including wild pea subspecies Pisum sativum syriacum (Boiss. et Noe) Berger (= P.s. humile Boiss. et Noe) and P.s. elatius (Bieb.) Schmahl. (1). The screening of a world pea collection of the All-Union Plant Breeding Institute, Leningrad (VIR), as well as the analysis of data available from the catalogue of the Weibullsholm pea collection (3), revealed a high concentration of the Np allele in the Balkans, the Near East, and Middle Asia (Table 1). In contrast with the data of Dodds and Matthews (1), we found a low proportion of Np in accessions originating from Ethiopia. All seven examined accessions of P.s. elatius and four of the five studied accessions of P.s. syriacum displayed the neoplastic pod phenotype under greenhouse conditions. The four studied accessions of Pisum fulvum Sibth. et Smith, lacked this character. It seemed to be highly improbable for such a common allele as Np, which is, moreover, characteristic to natural forms, to be deleterious. One could suppose that the feature appearing under artificial conditions is only a misfunction of a gene responsible for some beneficial property of a plant.

It was reported by Vilkova et al. (5) that certain outgrowths of the pod wall arise in response to oviposition of pea weevil (Bruchus pisorum L.), a common pest of garden pea. These authors stated that in accession VIR-1884 (Afghanistan) several days after oviposition a knob started to grow just under the area of oviposition until the eggs were removed. In 1990, we examined a number of peas grown in the VIR Experimental Station in Krymsk, Krasnodar Region, under a heavy pressure of the pest, and found that some of the accessions, namely, P.s. syriacum: VIR-7335 (Tajikistan), VIR-3727 (Turkey), and VIR-2514 (Syria); P.s. elatius: VIR-3115 (Italy); P.s. sativum: VIR-2422 (Morocco), VIR-4911 (Tibet), and VIR-6135 (Greece); P.s. transcaucasicum (Govorov) Makasch: VIR-2376 (Georgia); P.s. asiaticum Govorov: VIR-1884 (Afghanistan), did exhibit the described trait. The Bruchus ovipositions caused on the third to fifth day the growth of heavy amorphic knobs 2-4 mm in size which strikingly resembled the neoplastic pustules. After 6-8 days the outgrowths underwent necrosis and eventually fell away thus removing the eggs. (Sometimes numerous pustules of much smaller size appeared on the pods without any relation to eggs). However, a great proportion of the larvae managed to hatch earlier and penetrate into the pods, therefore, the strategy seemed not to be very effective. The size and number of formations varied among accessions being maximal in the P.s. syriacum forms. It was found that the seeds of accessions VIR-7329 and VIR-2376 later turned out to be the least infested by bruchid larvae among 30 tested accessions.

Weibullsholm			VIR		
Region	Np (%)	Studied	Region	Np (%)	Studied
North Europe <sup>1</sup>	2.1	190	North Russia	0	33
South Europe <sup>2</sup>	13.9	36	South Russia + Ukraine	4.3	23
Greece + Albania	63.0	98	Caucasus + Trans-Caucasic region	19.4	36
Near East <sup>3</sup>	32.5	40	Asia Minor + Palestine	28.9	38
			Balkans <sup>6</sup>	56.2	32
Ethiopia	2.4	83	Ethiopia	5.7	35
Middle Asia <sup>4</sup>	12.4	97	The Pamirs	16.7	24
Far East <sup>5</sup>	4.3	23	Afghanistan	34.6	26

Table 1. The proportion of accessions with allele *Np* in regional samples of pea accessions from the Weibullsholm and VIR collections.

<sup>1</sup> Czechoslovakia, Denmark, England, Estonia, France, Germany, Holland and Poland

<sup>2</sup> Bulgaria, Hungary, Italy, Portugal, Romania, Spain and Yugoslavia

- <sup>3</sup> Armenia, Georgia, Israel and Turkey
- <sup>4</sup> Afghanistan, Iran and Uzbekistan
- <sup>5</sup> China, Japan, Korea and Mongolia
- <sup>6</sup> Albania, Bulgaria, Greece and Yugoslavia

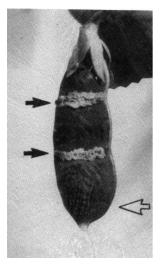


Fig. 1. The pod of an  $F_1$  hybrid between lines WL577 (*np*) and VIR-5797 (*Np*) treated with a 0.9% NaCl solution (white arrow - one can see the marks left by a glass capillary) and with a *Bruchus* individual homogenised in the solution (black arrows). The photo was taken on the fifth day after treatment. The dark tone of the pod is due to the presence of dominant genes *Pu* and *Pur* (determining a purple colouration of pods). The hybrid was heterozygous for these genes. The cells of the neoplastic formations are void of anthocyanin, thus confirming their epidermal nature that was questioned by Dodds and Matthews (1).

These observations allowed us to suppose that gene Np is associated with pest resistance in pea. To test the hypothesis we conducted the following experiment. A weevil individual was homogenized in 0.9% NaCl solution. The homogenate and the pure salt solution were applied to pods of two pea samples grown in a greenhouse: lines WL1238 (Weibullsholm collection), A783, and "Slow" (received from Dr N. Weeden), that do not exhibit a neoplastic phenotype, and the F<sub>1</sub> hybrid between line WL577 and VIR-5797 (Crete). Line VIR-5797 has the Np gene but its expression in the hybrid is very weak (sparse tiny pustles). It was important to use pea forms with weak expression of the neoplastic trait in order to discriminate natural outgrowths from induced ones. Three days after the treatment, those regions of the pod wall in the hybrid that were contacted with the beetle homogenate gave rise to heavy pustules (Fig. 1) while those contacted with the pure solution did not. The effect was the same irrespective of whether the pod epidermis remained intact or was injured by the glass capillary. No effect of treatment was observed in peas which lacked the Np allele. This indicates that the homogenate contains some factor which induces proliferation of epidermal cells provided the Np allele is present. This factor evidently should also be present in the liquid secreted by a beetle during oviposition. Investigation of the chemical nature of the inducing substance is currently in progress.

The present results indicate that the *Np* allele may provide a measure of resistance to attack by *Bruchus pisorum* by conferring on the pod epidermal cells the ability to form an outgrowth in response to oviposition. This also explains the high concentration of the gene in southern regions, where the pressure of the pest is especially high (4).

- 1. Dodds, K.S., and Matthews, P. 1966. J. Hered. 57:83-85.
- 2. Nuttal, V.W., and Lyall, L.H. 1964. J. Hered. 55:184-186.
- 3. The Weibullsholm Pea Collection, Weibullsholm Plant Breeding Institute, 1975.
- 4. Vasiliev, I.V. 1939. Vestnik Zashchity rastenii, No. 1 (20), p. 44-45. (in Russian).
- 5. Vilkova, N.A., Kolesnitchenko, L.I., and Schapiro, I.D. 1977. Methodical recommendations on revealing resistance of garden pea cultivars to pea weevil. Leningrad, VIR. (in Russian).