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# Inheritance and Expressivity of Neoplasm Trait in Crosses between the Domestic Pea (*Pisum sativum* subsp. *sativum*) and Tall Wild Pea (*Pisum sativum* subsp. *elatius*)

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**Abstract:** The Neoplasm trait in pea pods is reported to be due to the lack of ultraviolet (UV) light in glasshouse conditions or in response to pea weevil (*Bruchus pisorum* L.) damage. This pod deformation arises from the growth of non-meristematic tissue on pods of domesticated peas (*Pisum sativum* L. subsp. *sativum*). Neither expressivity, nor the effect of pea weevil on neoplasm in the tall wild pea (*P. sativum* L. subsp. *elatius* (M. Bieb.) Asch. & Graebn.), have been adequately studied. We aimed to study the expression and inheritance of neoplasm in the tall wild pea and crosses between domesticated and tall wild peas grown in the glasshouse (without pea weevils) and in the field (with pea weevils) under natural infestation conditions. Neoplasm was found in all pods in tall wild peas when grown in the glasshouse, while it was not detected on pods of field-grown plants despite heavy pea weevil damage. In inter-subspecific crosses between *P. sativum* subsp. *sativum* and *P. sativum* subsp. *elatius*, all $F_1$ plants had neoplastic pods, and the $F_2$ populations segregated in a good fit ratio of 3 (neoplasm): 1 (free from neoplasm) under glasshouse conditions, which suggests that neoplasm on pods of the tall wild pea was controlled by a single dominant gene. Expressivity of neoplasm in the progeny differed from parent to parent used in inter-subspecific crosses. There was no relationship between neoplasm and damage by pea weevil under heavy insect epidemics under field conditions. The neoplasm occurring under glasshouse conditions may be due to one or to a combination of environmental factors. Since wild peas are useful genetic resources for breeding programs aiming at fresh pea production that could be utilized under glasshouse conditions, negative selection could be considered in segregating populations.

**Keywords:** tall wild pea; *Pisum sativum* subsp. *elatius*; neoplasm; pea weevil; *Bruchus pisorum*; expressivity; inheritance

## 1. Introduction

The genus *Pisum* L. consists of the following species, subspecies and varieties: *P. sativum* L. subsp. *sativum* var. *sativum*, *P. sativum* L. subsp. *sativum* var. *arvense* (L.) Poir., *P. sativum* L. subsp. *elatius* (M. Bieb.) Aschers. & Graebn. var. *elatius*, *P. sativum* L. subsp. *elatius* (M. Bieb.) Aschers. & Graebn. var. *brevipedunculatum* Davis & Meikle, *P. sativum* L. subsp. *elatius* (M. Bieb.) Aschers. & Graebn. var. *pumilio* Meikle, *P. fulvum* Sibth. & Sm. and *P. abyssinicum* A. Br. [1–5]. The former two varieties are under cultivation as the garden pea and field pea, respectively; while *P. sativum* subsp. *elatius* and *P. fulvum* are wild species [3–5]. *P. sativum* subsp. *abyssinicum* is referred to as dekoko or Abyssinian pea and grown in eastern Africa and the Arabian Peninsula [3,6]. *P. sativum* subsp. *elatius*, known as the tall wild pea, is native to the Europe–Mediterranean region, the Balkans, the Crimean and Caucasian region, the Middle East and northwest Asia [3,5,7], whereas *P. fulvum* is limited to the Middle East [3] and Turkey [1].

Domesticated peas are not only important protein sources in the world but also essential rotation crops, especially in cereal-based cropping systems for sustainable agriculture due to fixation of atmospheric nitrogen [8–11]. The field pea is grown as a fodder crop for animal feeding, while the Abyssinian and garden peas are grown for dry seeds as a food legume. Dried seeds of the domesticated pea had a production of 13.5 million t from an area of 7.9 million ha with an average seed yield of 1718 kg per ha, while 21.2 million t of green peas as a vegetable were produced from 2.7 million ha with an average yield of 7735 kg per ha in the world in 2018 [12]. The statistics given for the domesticated pea place it in the second rank among food legumes based on production quantity [12].

Garden peas are often used as green peas (frozen and canned) or as fresh vegetables for fresh pods [3,13], but disease and insect damage on pea pods are undesirable for fresh use. Some genotypes of the *Pisum* species, when deprived of ultraviolet (UV) light in glasshouse conditions, form neoplasm on the pods. Neoplasm emerges as a response to the lack of UV light [14,15] on the surface of young pods with the growth of non-meristematic tissue. It is also stated that neoplasm is triggered by the pea weevil (*Bruchus pisorum* L.) [16,17]. Neoplasm found in domesticated plants occurs as a result of mutation, and a smooth pod without neoplasm is governed by a recessive "*np*" gene, while neoplasm is controlled by a single dominant gene "*Np*" [14,15]. Pods with neoplasm are not preferred by consumers due to the unpleasant image of the pods.

Expressivity, which is the degree of phenotypic visibility of neoplasm in the progeny in domesticated peas, is influenced not only by environmental factors such as lack of UV light and the pea weevil [16–18], but also by dominant genes responsible for homozygous (*Np/Np*) or heterozygous (*Np/np*) mutations [17,19]. Neoplasm has also been reported to occur in wild species such as *P. elatius* M. Bieb. and *P. humile* Boiss. & Noe. when grown under glasshouse conditions [15]. However, the expression and inheritance of neoplasm have not been adequately studied in progeny obtained from inter-subspecific crosses between *P. sativum* subsp. *sativum* and *P. sativum* subsp. *elatius* species. Since wild peas are sources for improvement of biotic and abiotic stresses in pea breeding programs, it is important to understand the inheritance and expressivity of an undesirable characteristic such as neoplasm in these germplasms. Therefore, the aims of this study were (i) to study the inheritance of neoplasm and (ii) to determine the expression of neoplasm in progeny derived from inter-subspecific crosses between *P. sativum* subsp. *sativum* and *P. sativum* subsp. *elatius* species.

## 2. Materials and Methods

### 2.1. Plant Materials

Three accessions (AWP 442, AWP 449 and AWP 451) of the tall wild pea (*P. sativum* subsp. *elatius* (M. Bieb.) Asch. & Graebn.) formed neoplasm (*Np*), while four accessions (ACP 13, ACP 14, ACP 20 and ACP 773) of domesticated species (*P. sativum* L. subsp. *sativum*) were free from the neoplasm (*np*) trait when grown in a glasshouse.

The original numbers of AWP 442, AWP 449 and AWP 451 were IG 52442, IG 52459 and P 51 (ICARDA, The International Center for Agricultural Research in the Dry Areas, germplasm collection), respectively. AWP 442 was reported to be resistant to the pulse beetle (*Callosobruchus chinensis* L.), while AWP 449 was susceptible [20]. The important traits of the *Pisum* subspecies are detailed in Table 1. Crosses' populations (ACP 13 × AWP 442, ACP 773 × AWP 451, ACP 14 × AWP 449 and ACP 20 × AWP 442) were used for neoplasm observations under glasshouse conditions, whereas 210 accessions including the parents of these crosses were screened for neoplasm and pea weevil under field conditions.

**Table 1.** Important traits of *Pisum* subspecies used in inter-subspecific crosses.

| Subspecies | Accessions | Landrace/Wild | *Np* in Field | *Np* in Glasshouse (*Np*/*np*) | Flower Color | 100-Seed Weight (g) | Tolerance to Cold | Resistance to Seed Beetle | Resistance to Pea Weevil |
|---|---|---|---|---|---|---|---|---|---|
| *P. s.* ssp. *sativum* | [1] ACP 13 | Landrace | No | *np* | Pink | 23.5 | Medium | Susceptible | Susceptible |
| *P. s.* ssp. *sativum* | ACP 14 | Landrace | No | *np* | Pink | 29.7 | Medium | Susceptible | Susceptible |
| *P. s.* ssp. *sativum* | ACP 20 | Landrace | No | *np* | White | 34.1 | Medium | Susceptible | Susceptible |
| *P. s.* ssp. *sativum* | ACP 773 | Landrace | No | *np* | White | 28.9 | * | * | * |
| *P. s.* ssp. *elatius* | [2] AWP 442 | Wild | No | *Np* | Lilac purple | 9.8 | Tolerant | Resistant | Susceptible |
| *P. s.* ssp. *elatius* | AWP 449 | Wild | No | *Np* | Lilac purple | 10.6 | Tolerant | Susceptible | Susceptible |
| *P. s.* ssp. *elatius* | AWP 451 | Wild | No | *Np* | Lilac purple | 11.2 | * | * | * |

[1] Akdeniz University cultivated *Pisum*, [2] Akdeniz University wild *Pisum*, * not evaluated.

## 2.2. Field Screening for Pea Weevil

A total of 210 accessions of *Pisum* ssp. was evaluated for resistance to pea weevil (*Bruchus pisorum* L.) under field conditions for seven years, from 2014 to 2020 at Antalya (30°38′ E, 36°53′ N and 51 m above sea level), Turkey. ACP 13, ACP 14, ACP 20, ACP 773, AWP 442, AWP 449 and AWP 451 were grown every year. Incidence of pea weevil for each accession was scored using a 1–9 visual scale, based on percent damage under natural insect infestation in the field, where 1 = free from any damage by pea weevil, 9 = damage in more than 91% of seeds (Table 2). According to the scale, the accessions having a rate between 1 and 4 were considered to be resistant and those having a rate between 5 and 9 were regarded as susceptible. Damage evaluation of the infested seeds for pea weevil in field trials was carried out after harvesting and threshing. One hundred seeds selected randomly from four plants were assessed for seed damage evaluation in each accession and replication. Mean of percent seed damage was used for data analyses. Percent seed damage was used for resistance to pea weevil by Teshome et al. [21] and Aznar-Fernandez et al. [22].

**Table 2.** A visual 1–9 scale for resistance to pea weevil in *Pisum* species under field conditions.

| Score | Response to Pea Weevil | Damages on Seeds |
|---|---|---|
| 1 | Very Highly Resistant | Free from any seed damage after careful observation |
| 2 | Highly Resistant | Damage present in 1 to 10% of the seeds |
| 3 | Resistant | Damage present 11 to 20% of the seeds |
| 4 | Moderately Resistant | Damage present in 21 to 30% of the seeds |
| 5 | Less susceptible | Damage present in 31 to 40% of the seeds |
| 6 | Moderately susceptible | Damage present in 41 to 50% of the seeds |
| 7 | Susceptible | Damage present in 51 to 70% of the seeds |
| 8 | Highly susceptible | Damage present in 71 to 90% of the seeds |
| 9 | Very highly susceptible | Damage present in more than 91% of the seeds |

## 2.3. Plant Sowing and Growing

In the glasshouse, which was at the same location as the field experiments, seeds of the parent plants were sown from row to row at 100 cm and from plant to plant at 20 cm in 2016, and the same sowing norm was used for progeny in the glasshouse. Plants were watered with a drip irrigation system and weeds were removed by hand.

In the field, the experiments were conducted as a randomized complete block design with three replications from October to June. Plots were arranged as a single row of 2 m length with inter-row spacing of 50 cm and intra-row spacing of 10 cm. Plants were grown under rainfed conditions,

and weeds were cleaned by hand during the seedling stage. In addition, harvesting and threshing were done manually.

The same soil was used in glasshouse and field. According to the soil analysis results of the experimental field, the amount of organic matter and nitrogen in the soil was low and the soil texture was loam. Although the plant nutrient elements in the soil were generally balanced, it had been detected to be low in iron and zinc [23].

### *2.4. Climatic Conditions in Field and Glasshouse*

The field experiments for pea weevil resistance screening were conducted from October to June in the years between 2014 and 2020. During this period, long-term average monthly maximum temperatures were recorded as 32.7 °C, 26.3 °C, 21.5 °C, 19.4 °C, 23.3 °C, 24.3 °C, 29.3 °C, 31.9 °C and 41.4 °C from October to June, respectively. Long-term average monthly minimum temperatures were recorded as 14.0 °C in October, 10.3 °C in November, 5.5 °C in December, 3.1 °C in January, 6.8 °C in February, 5.7 °C in March, 11.6 °C in April, 14.2 °C in May and 19.4 °C in June. The long-term average total rainfall was 26.5 mm in October, 83.3 mm in November, 145.7 mm in December, 207.1 mm in January, 66.7 mm in February, 119.2 mm in March, 25.0 mm in April, 31.6 mm in May and 18.6 mm in June (Figure 1A). Weather conditions were also given by Kivrak et al. [24].

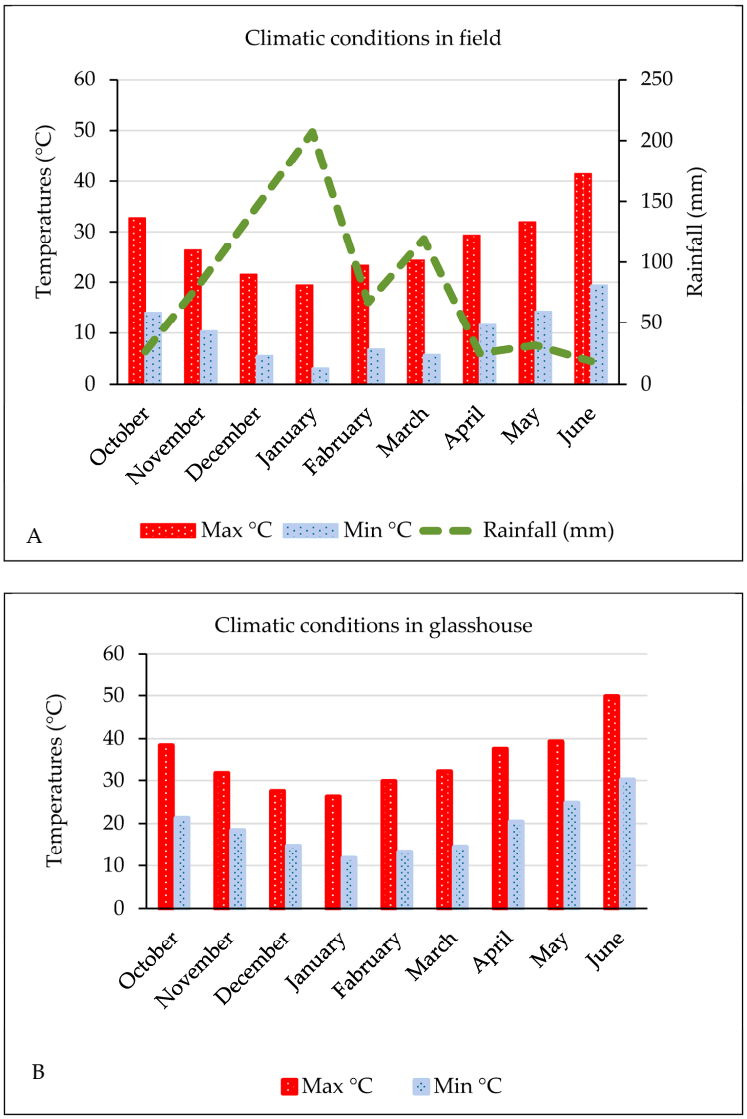


**Figure 1.** Long-term climatic conditions in field (**A**) and glasshouse (**B**) from 2016 to 2020.

Under glasshouse conditions, average monthly maximum temperatures from 2016 to 2020 were recorded as 38.4 °C in October, 31.7 °C in November, 27.5 °C in December, 26.2 °C in January, 29.8 °C in February, 32.1 °C in March, 37.6 °C in April, 39.3 °C in May and 49.8 °C in June. The monthly average minimum temperatures were recorded as 21.3 °C in October, 18.4 °C in November, 14.7 °C in December, 11.8 °C in January, 13.3 °C in February, 14.5 °C in March, 20.4 °C in April, 24.8 °C in May and 30.2 °C in June (Figure 1B).

### 2.5. Evaluation of Neoplasm

Presence (*Np*) and absence (*np*) of neoplasm on pods of accessions was checked weekly starting with pod formation until harvest under both glasshouse and field conditions. In glasshouse conditions, the density of neoplasm on pods of accessions and $F_1$ to $F_3$ progeny was visually assessed in accordance with previously reported dominant homozygous (*Np/Np*) or heterozygous (*Np/np*) alleles [14,17,19].

### 2.6. Expressivity of Neoplasm Trait

In the $F_2$ population, expressivity (E) of neoplasm was calculated according to the formula proposed by Yasar et al. [25]. According to this,

$$\text{E (\%)} = (\text{No of } Np \text{ plants/No of total plants}) \times 100 \quad (1)$$

### 2.7. Plant Crosses and Progeny

In the spring of 2016 on campus, the following inter-subspecific crosses were made between ACP 13 (*P. sativum* subsp. *sativum*) and AWP 442 (*P. sativum* subsp. *elatius*), ACP 14 (*P. sativum* subsp. *sativum*) and AWP 449 (*P. sativum* subsp. *elatius*), ACP 20 (*P. sativum* subsp. *sativum*) and AWP 442 (*P. sativum* subsp. *elatius*), and between ACP 773 (*P. sativum* subsp. *sativum*) and AWP 451 (*P. sativum* subsp. *elatius*). That is, domesticated peas that were free from neoplasm were used as female parent (♀), while wild tall peas having neoplasm were used as male parent or pollen donor (♂). Progeny derived from the inter-subspecific cross between ACP 13 and AWP 442 were advanced from $F_1$ to $F_3$ as one generation per year. Only one of the inter-subspecific crosses between ACP 13 (*np*) and AWP 442 (*Np*) was advanced to $F_3$ from 2016 to 2019, whereas the inter-subspecific crosses between ACP 773 (*np*) and AWP 451 (*Np*) were advanced to $F_2$ in 2020, while the inter-subspecific crosses between ACP 14 (*np*) and AWP 449 (*Np*), and between ACP 20 (*np*) and AWP 442 (*Np*), were used for confirmation and advanced to $F_1$. From $F_1$ to $F_3$, progeny were advanced as single plant progeny grown in the same glasshouse.

### 2.8. Data Analysis

Progeny having neoplasm were counted in segregating populations derived from inter-subspecific crosses between ACP 13 (*np*) and AWP 442 (*Np*), and also between ACP 773 (*np*) and AWP 451 (*Np*). The chi-square test ($\chi^2$) [26] was used to test the expected 3:1 ratio of segregation in the $F_2$ population:

$$\chi^2 = \frac{(O-E)^2}{E} \quad (2)$$

where *O* and *E* are the observed and expected values, respectively. The data on percent seed damage were subjected to analysis of variance (ANOVA) using SPSS 22.0 software (SPSS: Chicago, IL, USA).

## 3. Results

### 3.1. Relationships between Neoplasm and Pea Weevil

The screening test for seed damage indicated that three of the accessions were found to be very highly resistant with a score of one (free from any seed damage), three were highly resistant with a score of two (seed damage of 1–10%), and forty-four accessions were resistant with a score of three (seed damage of 11–20%). On the other hand, 78 accessions had considerable seed damage with a score

of four (seed damage of 21–30%). ACP 13, ACP 14, ACP 20 and AWP 442 accessions were found to be moderately susceptible (seed damage of 31–40%), while AWP 449 was susceptible with a score of six (seed damage of 41–50%) under natural pea weevil infestation conditions in the field (Figures 2 and 3). Significant differences among the accessions were determined for percent seed damage at a probability level of $p \leq 0.05$ but accession-by-year interaction was not significant ($p \leq 0.05$), which means that the accessions exhibited stable reaction over years regarding seed damage.

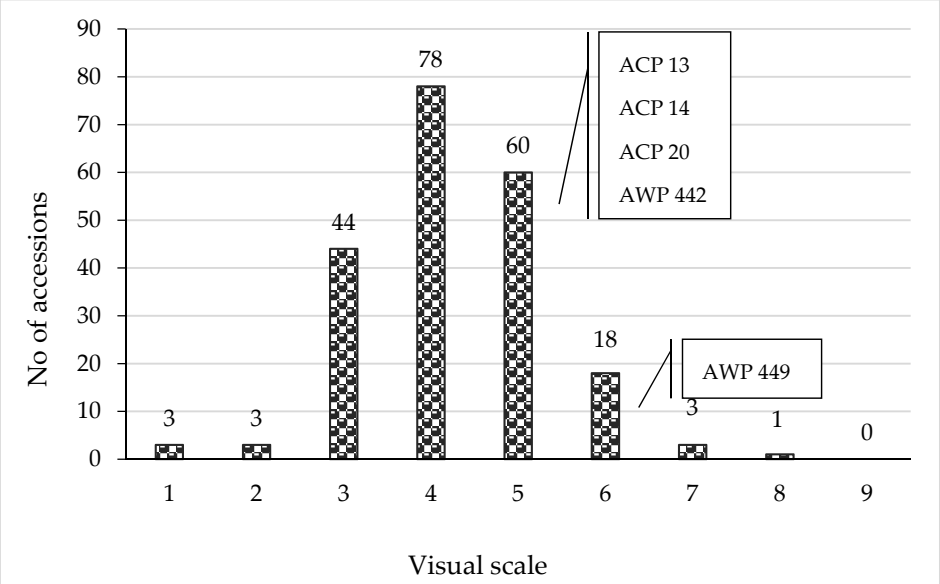


**Figure 2.** Resistance classes of pea accessions for the pea weevil based on a visual scale of 1–9 under field conditions.

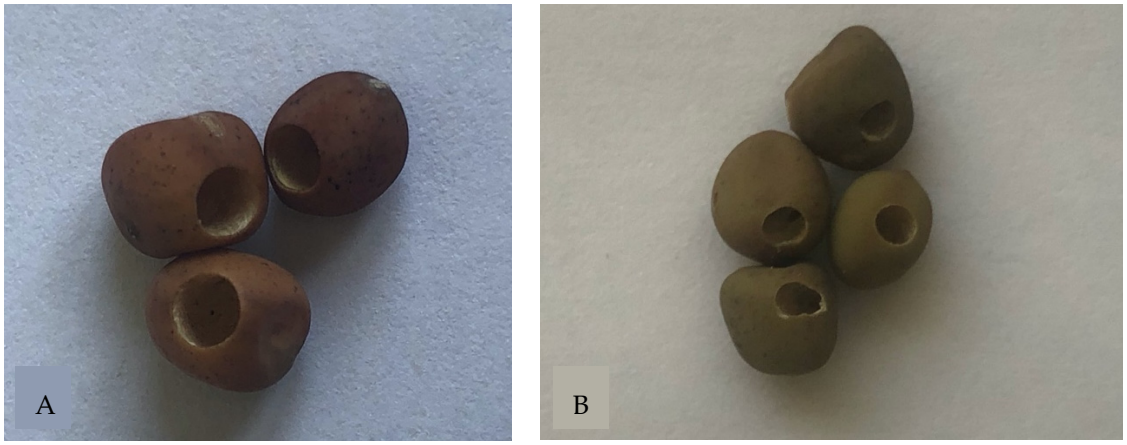


**Figure 3.** Seed damage holes of pea weevil in AWP 442 (**A**) and AWP 449 (**B**) under field conditions.

Accessions (ACP 13, ACP 14 and ACP 20) of *P. sativum* subsp. *sativum* were free from neoplasm both in field and glasshouse conditions (Figure 2 and Table 1). Despite the natural pea weevil infestation under field conditions, neoplasm did not occur on pods of accessions (AWP 442 or AWP 449) of *P. sativum* subsp. *elatius*, indicating no relationship between neoplasm and presence of pea weevil (Figures 2 and 3A,B). However, neoplasm on pods of the tall wild pea was observed under glasshouse conditions (Figure 4). In addition, neoplasm occurrence was frequent in young pods, while density of neoplasm decreased in mature pods (Figure 4A,B). Neoplasm was also observed on young and matured pods in the natural habitat of the tall wild pea in the Taurus Mountains, Antalya, Turkey (30°24′ E, 36°52′ N and 1071 m above sea level) during expeditions from May to July of 2020 (Figure 4C,D).


**Figure 4.** Neoplasm on a young pod (**A**) and two mature pods (**B**) of a progeny derived from inter-subspecific crosses between *P. sativum* subsp. *sativum* (ACP 13) and *P. sativum* subsp. *elatius* (AWP 442) in the $F_2$ population under glasshouse conditions in 2019. Neoplasm on a young (**C**) and mature pod (**D**) of a tall wild pea (*P. sativum* subsp. *elatius*) in its natural habitat in the Taurus Mountains, Antalya, Turkey in 2020.

### 3.2. Expressivity of Neoplasm

Phenotypic occurrence of neoplasm (expressivity) in the $F_2$ population obtained from inter-subspecific crosses between ACP 13 (*P. sativum* subsp. *sativum*) and AWP 442 (*P. sativum* subsp. *elatius*) was 77.2% (Table 3). In inter-subspecific crosses between ACP 773 (*P. sativum* subsp. *sativum*) and AWP 451 (*P. sativum* subsp. *elatius*), expressivity was found to be 67.9% (Table 3). However, no difference was seen between dominant homozygous (*NpNp*) or heterozygous (*Npnp*) progeny in $F_2$ and $F_3$ populations, while differences in density of neoplasm were observed among the pods of a progeny. Expressivity of neoplasm was found to be higher in inter-subspecific crosses between ACP 13 (*P. sativum* subsp. *sativum*) and AWP 442 (*P. sativum* subsp. *elatius*) than that in inter-subspecific crosses between ACP 773 (*P. sativum* subsp. *sativum*) and AWP 451 (*P. sativum* subsp. *elatius*), as shown in Table 3.

**Table 3.** Expressivity of neoplasm in $F_2$ populations derived from inter-subspecific crosses between *P. sativum* subsp. *sativum* and *P. sativum* subsp. *elatius*.

| Inter-Subspecific Crosses | Expected Ratio of Neoplasm | Observed Neoplasm Progeny | Expressivity (%) |
|---|---|---|---|
| ACP 13 (*np*) × AWP 442 (*Np*) | 3/4 (82.5) | 85 | 77.2 |
| ACP 773 (*np*) × AWP 451 (*Np*) | 3/4 (98.3) | 89 | 67.9 |

### 3.3. Inheritance of Neoplasm in P. sativum subsp. elatius

A total of 11, 12 and 15 progeny of three $F_1$s obtained from inter-subspecific crosses between ACP 13 (*P. sativum* subsp. *sativum*) and AWP 442 (*P. sativum* subsp. *elatius*), ACP 14 (*P. sativum* subsp. *sativum*) and AWP 449 (*P. sativum* subsp. *elatius*), ACP 20 (*P. sativum* subsp. *sativum*) and AWP 442 (*P. sativum* subsp. *elatius*), and between ACP 773 (*P. sativum* subsp. *sativum*) and AWP 451 (*P. sativum* subsp. *elatius*), was produced, respectively. All $F_1$ plants had neoplasm on their pods.

There were no differences of neoplasm on the pods of both inter-subspecific crosses in $F_1$ plants. In the $F_2$ population derived from the inter-subspecific cross between ACP 13 (*P. sativum* subsp. *sativum*) and AWP 442 (*P. sativum* subsp. *elatius*), neoplasm was observed on pods of 85 progeny, while 25 progeny were free from neoplasm. In inter-subspecific cross between ACP 773 (*P. sativum* subsp. *sativum*) and AWP 451 (P. *sativum* subsp. *elatius*), neoplasm was seen in 89 of 131 progeny. Based on the chi-square test, the segregations in both $F_2$ populations were found to have a good fit ratio of 3:1 (Table 4).

**Table 4.** Chi-square ($\chi^2$) analyses of neoplasm (*Np*) vs. free-from-neoplasm (*np*) progeny derived from inter-subspecific crosses between *P. sativum* subsp. *sativum* and *P. sativum* subsp. *elatius*.

| Inter-Subspecific Crosses | Phenotype of $F_{1s}$ | $F_2$ | | | | |
|---|---|---|---|---|---|---|
| | | No of Plants | Observed | Expected | $\chi^2$ | *p* |
| ACP 13 × AWP 442 | *Np* | 85<br>25 | *Np*<br>*np* | 3:1 | 0.3 | 0.9-0.1 |
| ACP 773 × AWP 451 | | 89<br>42 | *Np*<br>*np* | 3:1 | 3.5 | 0.9-0.1 |

$F_{1s}$: First filial generation, $F_2$: Second filial generation, *p*: Probability value.

### 3.4. Relationships between Neoplasm and Climatic Conditions

Maximum and minimum temperatures were higher in the glasshouse than those of the field conditions (Figure 1). Although light intensity was not recorded, neoplasm occurred on pods under the glasshouse conditions and pods in plants that are shaded under natural habitat conditions of the tall wild pea.

## 4. Discussion

ACP 13, ACP 14, ACP 20 and AWP 442 were moderately susceptible to pea weevil with seed damage of 31–40%, whereas AWP 449 had seed damage of 41–50% under natural pea weevil infestation conditions in the field (Figures 2 and 3). On the other hand, some accessions of *P. fulvum* were very highly resistant to pea weevil (Figures 2 and 3), as reported in previous studies [22,27–30].

Although neoplasm was observed on pods of AWP 442 and AWP 449 accessions of *P. sativum* subsp. *elatius* in the glasshouse, it did not arise under field conditions even when there was pea weevil damage on pea pods (Table 1, Figures 3 and 4). Neoplasm in peas with the *Np* gene has been reported to occur under glasshouse conditions with reduced UV light, as well as on pods that are shaded under natural habitat conditions [31]. Our findings on the shaded pods in the glasshouse and natural habitats are in agreement with those of Snoad and Matthews [31]. Although there is no clear evidence as to whether there is resistance to pea weevil in plants with the *Np* gene, it has been reported that pea weevils spend more time during oviposition in pods with neoplasm than in non-neoplastic pods [32], and that pea weevils prefer to oviposit in pods with neoplasm less than in non-neoplastic pods [21,30,33]. Neoplasm was not only reported by Nuttal and Lyall [14] in domesticated peas (PI 206988, PI 244219 and PI 261668), but the occurrence of neoplasm in wild *Pisum* species including *P. humile* Mill., *P. elatius* and *P. fulvum* was also stated by Dodds and Matthews [15]. Additional accessions of field pea (*P. sativum*) (32433A, 203084A, 235899A, 237065A, 226037A, 226037B, 226037C, 226037D and 226037E) having neoplasm were revealed by Teshome et al. [18]. In addition to these neoplastic peas, some accessions of *Pisum*, including *P. elatius* and *P. sativum* originating from different countries, were reported to

express neoplasm on pods when plants were grown in the glasshouse [16]. Neoplasm was stated to be induced by light quality (lack of UV light) and started to appear on the surface of young pods of peas with the growth of non-meristematic tissue [14,15]. It was also reported to be related to the pea weevil or bruchins production [16–18,22,30–36]. Neoplasm formation occurs due to light quality such as insufficient UV light or as an antibiosis mechanism in pea weevil resistance [17,33]. Aznar-Fernandez et al. [22] revealed that neoplasm was formed in the pods of the P669 accession without pea weevil eggs in their field trials for resistance to pea weevil in peas. Neoplasm did not occur in field conditions despite pea weevil damage in our findings. On the other hand, it occurred without any pea weevil damage in the glasshouse in the present study. Neoplasm in these accessions of *P. sativum* subsp. *elatius* depended on reduced light in the glasshouse and shadow in natural habitat in the Taurus Mountains (Figure 4). In addition, there may be a relationship between occurrence of neoplasm and high temperature because temperatures during pod formation stages were higher (about 20 °C) in the glasshouse than those of the field conditions (Figure 1). Thus, temperature deserves attention in future studies. Teshome et al. [21] and Aznar-Fernandez et al. [22] emphasized that neoplasm formation was observed more frequently under glasshouse conditions. Aznar-Fernandez and Rubiales [37] did not distinguish whether the reduced pea weevil infestation was due to neoplasm that reduced ovulation or the inhibition of penetration on the pods. With the application of bruchin to pea pods, the expression of *CYP93C18*, a putative isoflavone synthase gene, increased, followed by an increase in the level of pisatin, an isoflavone phytoalexin [34]. Neoplasm formation on pods of the domesticated pea was negatively correlated with oviposition by pea weevil [30]. It was pointed out that wall thickness of the pod and trichomes on the pod of the domesticated pea might have affected the oviposition preference of the weevils [30].

Nevertheless, in the present study, neoplasm in accessions of *P. sativum* subsp. *elatius* was not triggered by pea weevil, indicating that the gene *Np* in these accessions could be different from those in previous studies [14,15,18]. Although neoplasm was related with pea weevil damage in previous studies [16–18,31,33], there was no relationship between neoplasm and pea weevil damage in accessions of *P. sativum* subsp. *elatius* (Table 1) in the present study. Doss et al. [17] outlined that peas having the *Np* gene were more resistant to pea weevil than others, and this was supported by Teshome et al. [18]. Pea weevil damage in *Np* accessions was found to be lower when compared to neoplasm-free ones, and so, a method of enhancing *Np* expression under field conditions via intercropping with sorghum and maize has been proposed, which can serve as part of an integrated pea weevil management strategy, especially for small-scale farming systems [18]. Based on the results found in the present study, neoplasm in accessions of the wild pea (*P. sativum* subsp. *elatius*) was symbolized as $Np^+$, as neoplasm in the tall wild pea could be a novel gene due to the fact that the gene was not affected by pea weevil (Table 1).

The expected number of neoplastic plants in $F_2$ progeny was 82.5 for ACP 13 (*P. sativum* subsp. *sativum*) × AWP 442 (*P. sativum* subsp. *elatius*), in agreement with the dominant single gene, while the observed number of neoplasms was detected as 85 progeny. Moreover, the expected number of neoplastic plants in ACP 773 (*P. sativum* subsp. *sativum*) × AWP 451 (*P. sativum* subsp. *elatius*) was 98.3, while the observed number of neoplasms was detected as 89 progeny (Table 3). Expressivity of neoplasm in $F_2$ progeny obtained from an inter-subspecific cross between ACP 13 (*P. sativum* subsp. *sativum*) and AWP 442 (*P. sativum* subsp. *elatius*) was found to be 77.2% (Table 3), while it was determined to be 67.9% in $F_2$ progeny obtained from a cross between ACP 773 (*P. sativum* subsp. *sativum*) and AWP 451 (*P. sativum* subsp. *elatius*) (Table 3). Expressivity of neoplasm in the tall wild pea was higher in the former inter-subspecific cross than that in the latter one (Table 3), explaining that it might be influenced by accessions used in inter-subspecific crosses or by year/environment. Aznar-Fernandez et al. [22] reported the effect of environment as the major factor in the development of neoplasm.

Neoplasm on pods of all progeny in $F_1$ populations obtained from inter-subspecific crosses between ACP 13 (*P. sativum* subsp. *sativum*) and AWP 442 (*P. sativum* subsp. *elatius*), ACP 14 (*P. sativum* subsp. *sativum*) and AWP 449 (*P. sativum* subsp. *elatius*), and between ACP 20 (*P. sativum* subsp. *sativum*) and AWP 442

(*P. sativum* subsp. *elatius*), was evident, indicating that neoplasm in these three *P. sativum* subsp. *elatius* accessions was dominant. In $F_2$ populations derived from crosses between ACP 13 (*P. sativum* subsp. *sativum*) and AWP 442 (*P. sativum* subsp. *elatius*), and between ACP 773 (*P. sativum* subsp. *sativum*) and AWP 451 (*P. sativum* subsp. *elatius*), progeny carrying neoplasm (*Np*) and free from neoplasm (*np*) were counted as 85 and 25 plants, respectively (Table 4), showing that neoplasm in accession (AWP 442) of *P. sativum* subsp. *elatius* was controlled by a single dominant gene (Table 4). Similar findings were also found in the inter-subspecific crosses among domesticated peas prior to the present study [14,15,18]. Expressivity and inheritance of neoplasm in the tall wild pea have not been adequately studied until now. This is the first study on expressivity of neoplasm in progeny derived from inter-subspecific crosses between *P. sativum* subsp. *sativum* and *P. sativum* subsp. *sativum* according to the available literature [38]. However, the linkage segment containing neoplasm (*Np*) in the domesticated pea was tagged as a part of LG (Linkage group) III by Weeden et al. [39] and Prioul et al. [40].

No differences in density of neoplasm on pods of homozygous (*NpNp*) or heterozygous (*Npnp*) $F_2$ progeny was found, while neoplasm was reported to be reduced in the heterozygous (*Npnp*) compared to the homozygous (*NpNp*) dominant progeny [15,18]. Since there was no difference in neoplasm density between the heterozygous (*Npnp*) and the homozygous (*NpNp*) dominant progeny, the neoplasm encoding gene in AWP 442, AWP 449 and AWP 451 accessions of *P. sativum* subsp. *elatius* could be novel (Figure 4). However, this needs to be confirmed by an allelism test, although findings in the natural habitat of the tall wild pea in the Taurus Mountains may support this (Figure 4C,D). Differences in density of neoplasm on pods of the same progeny were considered to be due to shade, since neoplasm in the natural habitat was found in shady places (Figure 4).

Wild species were pointed out to be important potential resources for breeding [41–43]. The pea's wild relatives, including *P. fulvum* and *P. sativum* subsp. *Elatius*, were reported to be resistant to many biotic and abiotic stressors such as pea weevil [28,29,44–46], powdery mildew [47], rust [48,49], fusarium wilt [50,51], ascochyta blight [52–54] and drought [55].

## 5. Conclusions

All $F_1$ plants had neoplastic pods, and the $F_2$ populations segregated in a good fit ratio of 3:1 under glasshouse conditions, which suggests that neoplasm on pods of the tall wild pea was controlled by a single dominant gene. The male parents carrying the neoplasm gene in AWP 442, AWP 449 and AWP 451 accessions of *P. sativum* subsp. *elatius* did not have different densities of neoplasm on pods, and the homozygous dominant (*NpNp*) progeny derived from inter-subspecific crosses did not have a distinct appearance from the heterozygous (*Npnp*) progeny, indicating that neoplasm in the tall wild pea could be under the control of a different gene or allele than the *Np* gene. No neoplasm occurred in AWP 442, AWP 449 and AWP 451 due to pea weevil damage in field conditions, but *Np* formation was observed in the same accessions and populations derived from them, although no pea weevil damage was present. It was understood that the neoplasm occurring in the accessions, and in the $F_2$ and $F_3$ populations, used in the study was not caused by pea weevil damage. The neoplasm occurring under glasshouse conditions could be due to one or to a combination of factors such as light intensity, humidity and temperature. Since wild tall pea accessions are potential genetic sources for breeding programs aimed at fresh pea production that could be utilized under glasshouse conditions, a negative selection scheme should be incorporated into breeding programs.

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