www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online; ISSN 1308-5301 Print 6/1 (2013) 18-21

Research article/Araştırma makalesi

Morphological features and inheritance of a dwarf -growth phenotype in a pea mutant line

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Abstract

A dwarf-growth pea mutant recovered after exposing seeds of *P. sativum* cv Iregi-5 to gamma-irradiation. The main characteristic of the plants was the shortened internode length. The shoot growth of the mutant was less than that of its parent. The line V/4 has shown to have single-gene recessive inheritance, characterized morphologically and for seed production. The early expression of the mutant phenotype makes it desirable characteristic for genetic- and physiological studies of the stem elongation and plant development.

Key words: Peas, *Pisum sativum*, Dwarf mutant, Inheritance, Internode length

1. Introduction

Pea (*Pisum sativum* L.), one of the most important food legume crops, provides excellent dietary components with health-promoting benefits and offers the important ecological advantage of contributing to the development of low input farming systems by fixing atmospheric nitrogen and further minimizing the need for external inputs when used as a break crop. The Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, developed a high-quality *P. sativum* mutant collection by mutagenizing a number of pea cultivars with chemical and physical agents. With a view to initiating an improvement program for pea mutant lines assessment, various field and laboratory studies were performed (Naidenova and Vassilevska-Ivanova, 2006; 2008; Vassilevska-Ivanova et al., 2008). During the course of field studies, a unique dwarf-growth mutant line was identified. The details of the mutant line and its inheritance are reported here for the first time.

It is widely accepted that dwarf phenotype in plants can be caused by the malfunction of biosynthesis or signaling pathways of different growth factors (Richards et al., 2001). The most intensively growth factors that modulate vegetative growth of shoots and other developmental processes are the gibberellins (GAs). Mutations blocking synthesis lead to a dwarf GA-responsive phenotype. Numerous such mutations are now known (Reid and Ross, 1993; Reid et al., 1996; Ross et al., 1997). For example, it was established that there are mutations at four loci that block active gibberellins in peas: *le* (Ingram et al., 1984), *na* (Ingram and Reid, 1987), *lh* (Swain et al., 1997) and *ls* (Ait-Ali et al., 1999). Several GA-synthesis mutations have been characterized at the molecular level and all are in structural genes. It is now clear some steps are controlled by gene families with distinct tissue specificity. Researches on auxin mutants are less well understood. Classical plant physiology experiments have indicated that indole-3-acetic acid (IAA) is required for normal stem elongation and for the inhibition of lateral bud outgrowth (Davies, 1995). Reports on developmental mutants with reduced level of IAA are rare, possibly due to the redundancy of IAA biosynthesis pathways (Normanly and Bartel, 1999; Symons et al., 2002), or because auxin deficiency may be lethal (Ngo et al., 2002; Kizil et al., 2010).

The objective of this study was to describe the morphological differences between normal (wild) plants and a dwarf-growth pea mutant, to determine the inheritance of the mutant trait and to evaluate the potential of the trait as a marker in genetic and physiological studies.

Abbreviations: GAs = gibberellins; IAA = indole-3-acetic acid; TSW = thousand seeds weight

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 BioDiCon. 183-0311

2. Materials and methods

Pea mutant V/4 was isolated in M_2 generation after irradiation of dry seeds of cultivar Iregi-5 with 100 Gy γ–rays (Co^{60}-source), at the Institute of Genetics, Bulgarian Academy of Sciences, by Dr. M. Vassileva. The mutant phenotype was verified in M_3 and in the next generations. *P. sativum* cv Iregi-5 is a mid-plant height, mid-season, anthocyaninless commercial variety. Initial cultivar and mutant plants were grown in the field at a rate of 100 plants/m^2. Conventional management practices, including insect control were used. Thirty randomly selected plants, both for mutant and their respective normal plants, were used for recording 20 variables, that characterize the mutant morphologically and also affect its production: days to first open flowers and to maturity (50 %), plant height (cm), plant height to the 1-st pod (cm), number of nodes to first pod, total number of nodes with expanded leaves on the main stem, number of pods (total, fertile and sterile) per plant, stem width at internode 8, petiole length at leaf 8, number and weight (g) of seeds per plant, number of fruitage nodes, pod length and pod width, number of seeds per pod, thousand seeds weight (TSW) (g), lodging resistance and protein content. Crude protein content (N x 6.25) in the seeds was calculated by the standard method. Node counts commenced from the first scale leaf as node 1. Internode 1 lays between nodes 1 and 2. Flowering time was taken as the days from sowing to appearance of the first open flowers.

Genetic behavior of the mutation was studied by making reciprocal crosses between the mutant and the parent genotype. We used chi-square test to determine the goodness -of - fit of the observed ratios to theoretical ratios (Rokitckij, 1967).

3. Results and discussion

Differences in stem growth between V/4 and Iregi-5 were visible soon after emergence and persisted during the entire growth period. The stem of V/4 was much shorter than that of Iregi-5 (Figure 1). The number of nodes was similar during the early stage, but V/4 had slightly fewer nodes than Iregi-5 during the late stage (Table 1).

The mutant V/4 had a pronounced apical dominance as Iregi-5; both, V/4 and Iregi-5 produced secondary shoots or lateral branches at nodes 1 and 2 rarely. Internode lengths at V/4 showed that the major factor accounting for the dwarfness was the short internode length (Figure 2 and 3). In both lines, the first flowers appeared around 74-75 days after planting and at the same nodes, 16 or 17. V/4 produced fewer and smaller pods and seeds than did Iregi-5 (Table 1). V/4 had 3.5 pods/ plant, whereas Iregi-5 bore 5.5 pods. The seed weight per plant and TSW were 2.78 g and 208.2 g for V/4 and 5.19 g and 219.4 g for Iregi-5, respectively. The seeds of V/4 and Iregi-5 were green and smooth, rarely wrinkled, and the cotyledons were green.

Figure 1. Plants of the dwarf-growth mutant line V/4 in the field.

The leaf surface was dull, dark-green and lusterless, and may be covered with a whitish bloom. The mutant plants had strongly reduced petiole length at leaf 8 by 63 %, and reduced stem width at internode 8 by 15 % when compared with the initial cultivar Iregi-5 but nevertheless; reduced tendency of the crop to lodging was not established. Furthermore, the stem tissue structure of the mutant differed from this of the initial cultivar: it is compact and rigid with good standing ability. The stem structure of the initial cultivar has clearly outlined thicker, polylamellate outer walls, arranged differently from those in the inner walls. As a whole, the pea stem is hollow with exception of part between 1 to 3, and the last two internodes, thereby reducing lodging resistance of the plants. It is quite possible, that the changes are response of the total wall material of the stem to the presence or absence of auxin. Similar changes of epidermal cell wall of pea stems were established during auxin-induced growth (Bret-Harte and Talbott, 1993). The reciprocal crosses between V/4 (dwarf-growth) and Iregi-5 (normal growth) gave F_1 plants with the normal traits only. In cross V/4 x Iregi-5, F_2 populations segregated into 165 normal and 50 dwarf-growth types (χ^2 = 0.35, P >0.50); and in reciprocal cross Iregi-5 x V/4 F_2 segregated in 195:59 (χ^2 = 0.47, P>0.25), respectively, thus fitting a monogenic 3:1 ratio. This indicates that the dwarfness is governed by a single recessive gene.

In the present study, we described a dwarf-growth mutant, which phenotypically was characterized by reduced plant stature. It is clear that the dwarf mutant has a dramatically altered phenotype with changes in a wide range of developmental traits.

Table 1. Morphological and productivity characteristics of a dwarf-growth mutant

Trait	cv Iregi-5	V/4
Days to first open flowers (50 %)	74	75
Days to maturity (50 %)	111	110
Plant height, cm	44.7 ± 0.83	11.4 ± 0.27***
Plant height to the first pod, cm	37.3 ± 0.50	8.9 ± 0.22***
Stem width internode 8, cm	0.47 ± 0.07	0.40 ± 0.06***
Petiole length leaf 8, cm	5.29 ± 0.08	1.93 ± 0.07***
No of nodes to the first pod	16.8 ± 0.17	15.7± 0.19***
Total number of nodes (with expanded leaves on the main stem)	21.0 ± 0.30	20.0 ± 0.22*
No of fruitage nodes	3.3 ± 0.21	2.9 ± 0.13
Pod length, cm	6.47 ± 0.09	6.13 ± 0.09*
Pod width, cm	1.58 ± 0.02	1.65 ± 0.02**
Total number of pods /plant	5.5 ± 0.29	3.5 ± 0.17***
No of fertile pods/plant	5.2 ± 0.22	3.2 ± 0.11***
No of sterile pods/plant	0.33 ± 0.11	0.30 ± 0.08
No of seeds/plant	23.7 ± 0.97	13.4 ± 0.54***
No of seeds/pod	4.51 ± 0.07	4.22 ± 0.12*
Weight of seeds/plant, g	5.19 ± 0.18	2.78 ± 0.11***
Thousand seeds weight (TSW), g	219.4	208.2
Protein content, %	26.95	25.85
Mean lodging score•	5	2-3

•Scale:1 = erect; 5 = prostrate; *P< 0.05, **P< 0.01, ***P< 0.001, based on t-test

Figure 2. 9. 11 internodes of the cv Iregi-5 (left) and dwarf-growth mutant line V/4 (right)

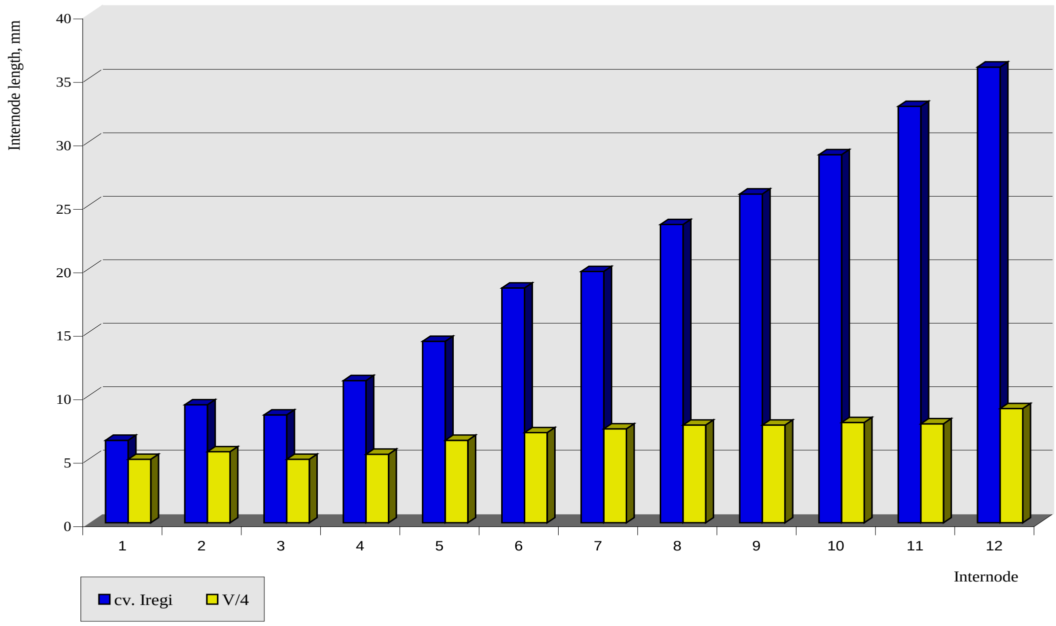

Figure 3. Internode length for the first 12 internodes of cv Iregi-5 and mutant line V/4.

Despite the severity of some of these changes, mutant plants are both viable and fertile, and show surprising vigor and standing ability. Together these characteristics make dwarf-growth V/4 a highly valuable research tool with the potential to provide many insights into the role of auxin and gibberellins in plant growth and development.

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(Received for publication 21 March 2011; The date of publication 01 April 2013)