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Recessive monogenic mutation in grain pea (*Pisum sativum*) that causes pyridoxine requirement for growth and seed production

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Abstract. A stable pyridoxine-deficient pea mutant was obtained by screening the M2 progeny of azide-treated *Pisum sativum* cv Pusa Harbhajan. The mutation is visible lethal. The isolation of pyridoxine-deficient mutant demonstrates directly that pea plants synthesize their own pyridoxine and that pyridoxine is an essential growth factor for pea plants. The mutant character is determined by homozygous recessive alleles, designated *pdx-1,* at a single locus. Pyridoxine-deficient plants are fertile and indistinguishable from the wild type if supplied exogenously with 2 mg of pyridoxine.

Keywords. *Pisum sativum* mutant; pyridoxine auxotroph; pyridoxineless pea mutant; inheritance of pyridoxineless mutation; pyridoxine-requiring plant mutant.

Introduction

Pea (*Pisum sativum,* dicot, 2n=14) is a grain legume crop plant of world-wide importance. It is also one of the most intensively studied plants in physiological, biochemical, genetic, molecular biological and breeding experiments. Pea has received attention as an experimental plant largely because it has large flowers, a self-pollinating sexual mechanism and a wide array of easily observable seed, seedling and adult plant phenotypes (Murfet, 1985). Several genetic markers have been located on each of its 7 chromosomes (Blixt, 1974). These criteria make pea a useful plant model for molecular genetic analysis of biosynthetic and developmental processes and organization and regulation of genes, and for developing new material for breeding superior varieties of pea and other crop plants. In this context diverse kinds of biochemical gene markers are required in pea to provide basis for applications of methods of molecular genetics (genetic engineering).

So far, two kinds of fertile biochemical (conditional lethal) mutants have been reported in pea. These are the chlorate-resistant, nitrate reductase deficient mutants (Warner *et al.,* 1982) and thiamine-deficient mutants (auxotrophs) (Kumar and Sharma, 1986). The available thiamine auxotrophs (Thi$^-$) belong to 3 complementation groups, analogous to *thiA, thiB* and *thiC* genes of *Escherichia coli* (Kumar and Sharma, 1986). A third kind of conditional lethal marker in pea, namely a pyridoxine (Pdx) mutant, in which auxotrophy is controlled by a pair of recessive alleles (*pdx-1*) at a single locus is reported here. This is the first report of Pdx auxotrophy in higher organisms. The genetic control of Pdx synthesis has been studied well only in *E. coli.*

Materials and methods

The Pdx$^-$ mutant described here was isolated in a mutagenization experiment.

Abbreviations used: Pdx, Pyridoxine; Thi, thiamine.

P.sativum cv Pusa Harbhajan is a dwarf, early-maturing, anthocyaninless, commercial variety with round white seeds. Seeds of Pusa Harbhajan were soaked in a 1 mM solution of sodium azide (NaN_3) at pH 4 for 18 h. After mutagen treatment, seeds were washed and sown in the Institute field. The resulting M_1 plants were grown to maturity and single plant seeds were harvested. A total of 552 M_2 progenies that became available were examined for abnormal phenotypes from seedling emergence stage to maturity and thus a Pdx^- mutant was identified. The mutant was multiplied and tested in subsequent seasons. Reciprocal crosses Pdx^- × wild type were made and studied to understand the inheritance of the mutation.

Results and discussion

Of the 552 M_2 progenies examined, two segregated for Thi deficiency mutation. Within a third M_2 progeny there was one plant which also had visible lethal phenotype like that of the Thi auxotrophs. It grew and produced 8 green leaves like a normal plant. Later it produced 3 yellowish leaves and a flower and stopped growing. The phenotype strongly resembled that of Thi mutants, but the plant failed to respond to application of Thi pyrophosphate. When a mixture of vitamins was applied, the plant resumed growth and produced a number of green leaves and flowers. A few flowers matured into seed-filled pods. Seeds were collected from the mutant plant and from the normal-looking plants of the M_2 line among which the mutant was found. They.were sown separately to obtain M_3 generation progenies. The seeds from the mutant plant produced identical plants. Mutant plants were also recovered as segregants in some M_3 progenies. After the mutant M_3 plants had started to produce yellow leaves, criss-cross pools made with 2 mg/ml of Pdx hydrochloride, Thi hydrochloride, riboflavine, biotin, nicotinic acid, myo-inositol, calcium pantothenate, folic acid and *p*-aminobenzoic acid were applied to the plants. Yellowing and arrest of growth were found to be suppressed by those pools that contained pyridoxine hydrochloride. Later it was found that application of about 2 mg of Pdx hydrochloride alone could cure the mutant plants of their deficiency symptoms. The mutant displayed a homozygous genotype by breeding true in M_4 and M_5 generations. Thus it was concluded that the mutant failed to synthesize Pdx and its initial normal growth was due to Pdx received by the developing embryos from the mother plant through placental tissue. It was found that the onset of yellowing in mutant plants correlated with the amount of Pdx applied to their mother plants. Mutant plants exhibited very tight phenotype when their mother plants had been applied about 100 μg of Pdx hydrochloride. In mutant plants having tight phenotype yellowing started in any of the first 5 leaves. It was also observed that the yellow leaves of mutant plants had strikingly narrower leaflets compared to those on normal leaves.

Genetic behaviour of the mutation was studied by making reciprocal crosses between the mutant and the parent wild-type plants. Table 1 gives the results of crosses. It was observed that (i) all the F_1 hybrid progeny plants had wild-type phenotype, and (ii) F_2 seedlings segregated for the wild type and mutant phenotypes in 3:1 ratio. The results indicated that the Pdx auxotrophy must be controlled by a single recessive gene. The allele has been designated *pdx-1*.

Table 1. Segregation of the *pdx-1* mutation in pea.

Genotypic description of cross	Number of plants		Expected ratio	χ^2	*P*
	Pdx^+	Pdx^-			
F_1 (+/*pdx-1*)	25	0			
F_1 (*pdx-1*/+)	22	0			
F_2 (+/*pdx-1*)	219	67	3:1	0·09	0·95–0·50
F_2 (*pdx-1*/+)	110	38	3:1	0·04	0·95–0·50

There are a number of implications of the present work, including the following, (i) It has been directly demonstrated that plants require Pdx for their growth and they make their own Pdx. Assessment of the dependence of plant growth and harvest index on Pdx synthetic ability will provide criteria for selection in breeding experiments of genotypes having optimum Pdx synthetic capability, (ii) The *in vitro* cultured cells, tissues and organs and gynoecia of *pdx-1* mutant plants can be employed as recipients for transfer of Pdx^+ linked genes of homologous or heterologous origin so that the rare recombinants could be selected under restrictive conditions, (iii) It will be possible to study and manipulate Pdx synthesis, absorption and transport and roles of Pdx in metabolism, (iv) Knowledge about the phenotype of Pdx^- mutant will permit isolation of more Pdx^- mutants in pea and other plants.

Besides pea, Pdx^- mutants are known in *E. coli* (Bachmann, 1983), *Salmonella typhimurium* (Sanderson, 1984) and *Neurospora crassa* (Perkins *et al.,* 1982). In *E. coli,* mutations in any of 5 different genes cause Pdx auxotrophy (Bachmann, 1983). The available information on bacteria, protozoa, fungi, and invertebrate and vertebrate animals indicate that Pdx is associated as a cofactor with a number of enzymes, some of which are known to be involved in the synthesis of several amino acids (Sauberlich, 1968; Snell and Haskell, 1971).

Although auxotrophe for essential vitamins, amino acids and nitrogenous bases of nucleic acids are among the most frequently isolated mutations in prokaryotes and lower eukaryotes, a very small number of such mutants have been isolated in plants (McCourt and Somerville, 1987; Last and Fink, 1988; Reddy and Kumar, 1988). The currently available tight fertile auxotrophe in crop plants of food value are: (i) the Thi-requiring mutants in tomato, *Lycopersicon esculentum* (Boynton, 1966a, b); grain pea, *Pisum sativum* (Kumar and Sharma, 1986); and barley, *Hordeum vulgare* (Kumar and Sharma, 1987; Reddy *et al.,* 1988); (ii) the praline auxotrophs in corn, *Zea mays* (Racchi *et al.,* 1981); and (iii) the Pdx-requiring mutant of grain pea (present study).

In conclusion, a Pdx^- mutant of *P. sativum,* designated *pdx-1* has been isolated. The *pdx-1* is a single-locus, recessive mutation inherited according to Mendelian segregation. The lethal phenotype of the mutant demonstrates that in plants Pdx is essential for growth. The mutant *pdx-1* provides a new system for studies in plant molecular biology.

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