CHAPTER IV

SUMMARY

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Lathyrus sativus is a minor pulse crop of India and is grown in the states of Madhya Pradesh, Bihar, Uttar Pradesh and Gujarat. The plant contains a non-protein neurotoxic amino acid β-N-Oxalyl diaminopropionic acid (ODAP) which leads to dreadful paralytic disease called neurolathyrism in humans. Inspite of this demerit, it is still grown because of the high protein contents of its seeds (28-32%) and drought resistant nature.

This demerit has prompted the attention of plant breeders to employ breeding procedures both conventional screening and mutation breeding for selecting Lathyrus lines with low ODAP content. However, these attempts have been either impractical or not very successful. The reason for this failure has been due to lack of understanding of biosynthesis of ODAP in Lathyrus sativus and related species. Presently there is a evidence of only one short lived intermediate (BIA) in the biosynthesis of diaminopropionic acid (DAPA) the immediate precursor of ODAP and for only enzyme responsible for oxylation of DAPA that gives rise to ODAP.

In the present work we have carried out experiments using both in vivo and in vitro systems on the synthesis and accumulation of ODAP. Such studies when carried under control nutritional and environmental conditions would be ideal for the understanding of the biosynthetic and degradative pathways of ODAP in this important legume. Cell culture system is ideal for carrying out such work.

I. IN VIVO STUDIES :

1. Nutritional factors play a major role in the accumulation of ODAP in seeds of L. sativus . During seedling growth of variety Bharuch, nitrogen and phosphate as potassium salts in the media increased the dry matter accumulation. Nitrate in particular was more effective in inhibition of ODAP synthesis in the seedlings. The osmotic stress to seedlings using mannitol at higher concentration (0.4 mM) also inhibited ODAP accumulation.

2. Effects of nutritional regime was carried out at whole plant level in sand cultures under green house. Nitrate nitrogen at lower concentrations (0.3mM) enhance the growth in terms of dry matter accumulation and inhibited ODAP synthesis by 50% over the control plants. The application of reduced nitrogen in the form of casein hydrolysate (CH) at 100 and 150 mg/l level also reduced the ODAP level of seeds. However, level higher than 200 mg/l had a lethal effect on the plant since the plant morphology was altered and flowering was inhibited. Thus it can be concluded that the nitrogenous fertilizers are helpful in lowering the ODAP level in the seeds.

3. Another approach to reduce the ODAP level was the foliar application of micronutrients. Four varieties were used for this experiment out of which P-24 and LSD-10 were low neurotoxin lines and Bh and LSD-2 were high neurotoxin lines. Cobalt (0.5 ppm) and molybdenum (20 ppm) when sprayed at flowering time improved the yield of seeds per plant. Moreover these treatments also reduced the ODAP level in the seeds of all the varieties.

Cobalt was more effective than molybdenum in the inhibition of ODAP accumulation. An interesting observation made in this work was that of a significant negative correlation between the total nitrogen content and ODAP content of the seeds. This indicates that the improvement of Lathyrus in terms of lowering ODAP would further benefit by elevating the total protein content of seeds.

Hence we conclude that improving the cultural practices in L. sativus by reducing water stress, and maintaining optimum macro and micro nutrient supply to the crop is necessary to bring down ODAP and to high protein levels in the seeds, thereby rendering the pulse less toxic to the human beings.

II. IN VITRO STUDIES :

1. An optimal medium in terms of basal salts, sucrose level and growth regulator supplements was standardisd for initiation and maintenance of callus and cell cultures of L. sativus.

2. It was found that ODAP is synthesized by cells grown in long term batch suspension cultures.

3. In general organic adenda of culture medium enhanced ODAP synthesis in cell suspension cultures. Cell cultures of various varieties exhibited genotypic variation in their response to organic addenda of culture media.

4. Cell suspension culture of variety Bharuch (Bh) was probed for a detailed study on effects of amino acid/amide addenda on synthesis of ODAP. Five amino acid/amide were administered

singly to the cells. The treatments had inhibitory effect on growth and degree of inhibition was variable with the type of amino acid/amide used. Growth and ODAP accumulation was inhibited to a greater extent by addition of glutamic acid, aspartic acid and glycine. Asparagine on the other hand stimulated growth as well as ODAP accumulation in cultures. A linear positive relationship was observed between the concentration of asparagine in the medium and accumulation of ODAP in cells suggesting asparagine as the precursor of ODAP.

5. Plant regeneration from long term batch suspension cultures is reported for the first time in L. sativus in this work. Modified B5 medium (MB5) supplemented with 1.5 μM 2,4-D and 0.3μM BAPwas induced development of shoot buds from callus derived from hypocotyl. Somatic embryogenesis was induced from immature embryo culture on MB5 media supplemented with 5 mg/l NAA or 2.5 mg/l 2,4-D, NAA being better than 2,4-D. Complete acclimatized plantlets were obtained from both systems. The embryogenic origin of regenerants was confirmed by histological studies.

6. Single cell somaclones were derived from plated cell cultures of L. sativus and were screened for low ODAP levels. Cell suspension was initiated from a somaclone that was lowest in ODAP content. This suspension was designated as LT-1 cell line (low toxin). Recurrent selection of somaclone for low ODAP was carried out using this procedure for four successive cycles. About 10% clones in each round showed ODAP levels considerably lower than average values. In this way at the fourth cycle of selection, somaclones were obtained with 2.5 fold reduction in

ODAP content (25-30 µg/g.dr.wt. in LT-4 lines vs 75-90 µg/g.dr.wt. of parental cells).

7. Plants were regenerated from LT-4 lines. ODAP level in vegetative parts of these plants was 124 and 135 µg/g.dr.wt. in the leaves and stem respectively. This was 30% of that in corresponding organs of seed derived plants. On the other hand, plants regenerated from unselected parental cell lines had values very close to the seed derived plants. Howerver, the data collected were of a small number of plants and R1 seeds could not be collected for further analysis. Hence further work is necessary before nature of variation of these clones is ascertained.

In conclusion, it can be said that plants can be regenerated from long term cultures of L. sativus either via organogenesis or somatic embryogenesis which is a pre-requisite for any crop improvement programme using in vitro selection. In the case such as Lathyrus where in vitro selection can not be done by applying selection pressure due to lack of information on the biosynthetic pathway of the compound in question, the approach of exploiting somaclonal variation for selecting low ODAP containing lines appears to be viable provided the trait is stable in successive generations.