Progress in isolation and purification of *Lathyrus sativus* breeding lines

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Introduction

Lathyrus sativus L. is the most important pulse crop in Bangladesh, where it is popularly known as "khesari". Among the pulses *L. sativus* occupies first position both in area and production and contributes about 35% of the total pulse production in Bangladesh. The national average yield of *L. sativus* is 750 kg/ha [(1)]. In Bangladesh the crop is generally grown in relay with *Aman* rice. However, it is also grown as a forage crop in some parts of the country where cattle are allowed to graze the crop.

Attempts have been made to ban its cultivation in Bangladesh due to its toxic effect in human beings. *Lathyrus sativus* seed contains a free amino acid known as β-N oxylaminoalanine (BOAA or ODAP) which can cause the neurological disorder called "lathyrism" [(5)]. Despite this problem, there is no alternative to *L. sativus* at present since it is a very hardy crop that tolerates adverse environmental conditions such as drought and excessive soil moisture. The crop produces yields with little or no inputs such as fertilizer and chemicals. Therefore, farmers choose to grow this crop extensively. The Pulse Research Centre (PRC) has taken initiatives to solve the neurotoxin problem, developing two varieties in 1995 and 1996 with high yield potential and lower BOAA content. The varieties and neurotoxin contents are found in Table 1.

Table 1. Two *L. sativus* varieties released by the Pulses Research Centre, Bangladesh and the mean BOAA (or ODAP) contents.

Varieties	BOAA content (%)
Barikhesari-1	0.29
Barikhesari-2	0.27

Lathyrus sativus is primarily a self-pollinated crop (see Ben Brahim *et al*. this issue) and breeding programmes have been followed accordingly. However, the natural outcrossing in this species is at a higher frequency than acceptable in a truly self-pollinated crop [(4)]. The extent of outcrossing varies according to the flower colour and has been recorded as high as 27.8% [(3)]. This high frequency of natural outcrossing has meant that the improvement programme will not be very reliable, as it will be difficult to maintain varietal purity in farmers' fields because of outcrossing with unimproved plants nearby. Therefore, breeding steps need to be taken to overcome the outcrossing problem by developing a visible morphological marker such as white, pink or red flowers in low toxin varieties. It has been observed in some studies that blue-flowered lines had relatively lower BOAA contents [(2)]. It may be possible to associate high level of BOAA with white, blue or red flowered lines. Therefore, effort should be made to initiate this research by isolating and purifying *L. sativus* breeding lines.

Research underway and results

The segregating generation developed so far in F_6, F_7 and other breeding lines have shown mixtures of flower colour. Therefore, the PRC have planned to purify the segregating lines along with other advanced lines with controlled pollination to develop homozygous pure lines based on flower colour.

In 1997-98, a total of 78 lines (7 lines from F_6, 28 lines from F_7, 20 lines from observation trial, 16 lines from preliminary yield trial and 7 lines from multilocation yield trial) were grown. Seeds from 10 plants from each of the 78 lines were harvested separately. In 1998-99, all these seeds were grown as plant to row at Pulses Research Centre, Ishurdi, Bangladesh with 50 x 50cm plant spacing. Initially 2 seeds were sown per hill and at seedling stage 1 plant per hill was maintained. At 1-2 flowering stage, each and every plant was observed for flower colour and a mixture of flower colour were noticed within each breeding line. The flower colour of a breeding lines was decided by seeing the majority of the plants having similar flower colour. Variable numbers of healthy plants within each breeding lines of similar flower colour were bagged with nylon mosquito nets of 5 cm x 30 cm size (mesh 6 holes per cm^2). A stick of 30 cm length was used to support the net and the plant. The flowers those already bloomed were removed from each plant during bagging. At maturity seeds from all the bagged plants were harvested and packed separately. Altogether 1779 plants of blue, pink, red and white flower colour were harvested in 1998-99 (Table 2).

In 1999-2000, all of those 1779 plants were grown again as plant to row in the similar way to the previous year. Plants were bagged with the same nylon mosquito net. It

was observed that most of the breeding lines had almost attained homozygosity. At the end of the season 1872 plants in total were harvested individually.

Table 2. The number of plants selected from each population in 1998-99.

Populations	**Flower colour**	**No. of plants harvested**
F_6	Blue/pink/red	159
F_7	Blue/pink	627
Observation trial	Blue/white/red	485
Preliminary yield trial	Blue	334
Multilocation yield trial	Blue/pink	174
Total		**1779**

These 1872 will be tested for their BOAA content. The low BOAA (or zero if found) content line(s) will be used as parents for crossing with the adapted cultivars or varieties to develop varieties for wider cultivation.

Meanwhile, this year (2000-2001) these 4 different flower coloured lines (white, blue, red and pink) have already been planted in the field for hybridization. They will be used to study the inheritance pattern of flower colour and be used simultaneously to develop varieties.

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