Development of mutagenesis technique for leguminous crops using heavy-ion-beam irradiation and screening of useful mutants

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Leguminous crops (soybean, mungbean, and peanut) are important sources of lipid and protein for the human diet. In Lao PDR, mungbean and soybean productions are well suited to local soil and temperature conditions, which include both wet and dry seasons. However, soybean and mungbean productions are currently operated on a small scale and at a household level depending on the local farmers' skill. There are several factors hampering the expansion of the production area and yield for both crops. Mungbean has an indeterminate flowering habit, which implies that it does not have a defined flowering period and will continue flowering as long as adequate soil moisture is provided. Consequently, harvesting mungbean is costly because of its long cultivation period and loss of grains from early-matured pods. On the other hand, most varieties of soybean are sensitive to the photoperiod. It is necessary to cultivate suitable varieties depending on the locations or seasons; otherwise, some varieties flower early, resulting in a low production yield. To overcome these difficulties, productions of mungbean with a determinate flowering trait and soybean with a non/less photosensitive trait are desired. Some mutants of soybean have been obtained with carbon-ion-beam irradiation.[1–3] In this study, to determine an efficient condition for mutant induction, we irradiated mungbean and soybean with heavy-ion beams at various absorbed doses and observed the effects on plant growth.

Dry seeds of mungbean (*Vigna radiate* 'Pakse') and soybean (*Glycine max* L. Merr 'ARC3') are packed into a rectangular plastic box or into a plastic petri dish in which a cork sheet is bedded to lay seeds in a single layer. Seeds of mungbean and soybean packed into the rectangular plastic box were irradiated with C ions (135 MeV/n) at doses of 0–150 Gy and 0–200 Gy, respectively. The LET of C-ion beams was controlled to 30 keV/μm. Seeds packed into the plastic petri dish were irradiated with Ar ions (160 MeV/n) at doses of 0–50 Gy. The LET of C-ion beams was controlled to 188 keV/μm. After irradiation, the seeds were sowed at an experimental field.

Table 1. Germination and growth of soybean (ARC3) irradiated by C-ion and Ar-ion beams at different doses.

Ion	Dose (Gy)	Germination rate (%)	Survival rate (%) *	Growth Index **
Control	0	90.0	90.0	1.00
C	25	63.3	63.3	0.99
	50	70.0	70.0	0.91
	75	70.0	56.7	0.82
	100	80.0	63.3	0.73
	125	80.0	23.3	0,50
	150	80.0	43.3	0.68
	200	80.0	16.7	0.26
Ar	5	83.3	83.3	0.88
	10	90.0	83.3	0.80
	20	90.0	76.7	0.65
	30	63.3	26.7	0.39

* Number of growing plants / 30 sown seeds

** Plant height /average height of control plants

Table 2. Germination and growth of mungbean (Pakse) irradiated by C-ion and Ar-ion beams at different doses.

Ion	Dose (Gy)	Germination rate (%)	Survival rate (%) *	Growth Index **
Control	0	100.0	83.3	1.00
C	25	96.7	80.0	0.93
	50	93.3	83.3	0.98
	75	80.0	80.0	0.95
	100	96.7	86.7	0.97
	125	96.7	93.3	0.83
	150	96.7	70.0	0.86
Ar	5	86.7	83.3	1.00
	10	90.0	70.0	0.92
	20	86.7	73.3	0.91
	30	90.0	90.0	0.92
	40	83.3	83.3	0.66
	50	63.3	63.3	0.54

Soybean showed sensitivity to both C-ion (in the range of 0–200 Gy) and Ar-ion (0–50 Gy) beams (Table 1). Although mungbean showed lower sensitivity, the growth rate was reduced to 0.83 after 125 Gy irradiation of a C-ion beam and to 0.54 after 50 Gy irradiation of an Ar-ion beam (Table 2). To optimize the mutation rate, we determined the minimal doses that yield a growth index appropriate for mutagenesis with heavy-ion beams. Therefore, we determined the appropriate absorbed doses for mutant selection as follows: for soybean, C-ion beams at 75 and 100 Gy, and Ar-ion beams at 10 and 20 Gy were used; for mungbean, C-ion beams at 125 and 150 Gy, and Ar-ion beams at 30 and 40 Gy were used. Among the irradiated soybean plants, some male sterile plants were found. The highest percentage of male sterile plants was 67% after Ar-ion-beam irradiation at 20 Gy, followed by 33%, 17%, and 17% after C-ion-beam irradiation at 150, 125 and 100 Gy, respectively. On the other hand, there were no male sterile plants among mungbean plants.

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