Notas Científicas

Variability of ricin content in mature seeds of castor bean

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Abstract – The objective of this work was to evaluate ricin concentration in castor bean seeds (*Ricinus communis*) of 20 accessions from the Banco de Germoplasma de Mamoneira of the Embrapa Algodão, Campina Grande, PB, Brazil, using the Enzyme Linked Immunosorbent Assay. Significant differences were observed among accessions. BRA 3271 had the highest ricin concentration in seeds (32.18 ng μg^{-1}), and BRS Paraguaçu had the lowest (3.53 ng μg^{-1}). There is the possibility of selecting genotypes with different ricin concentrations, which can be used according on the interest of the breeding programs.

Index terms: *Ricinus communis*, endosperm, germplasm bank, seed protein.

Variabilidade no teor de ricina em sementes maduras de mamona

Resumo – O objetivo deste trabalho foi avaliar a concentração de ricina em sementes de mamona (*Ricinus communis* L.) de 20 acessos do Banco de Germoplasma de Mamoneira da Embrapa Algodão, por meio de ensaio imunoabsorvente de ligação de enzimas. Foram observadas diferenças significativas entre os acessos. O BRA 3271 apresentou a maior concentração de ricina nas sementes (32,18 ng μg^{-1}), e o BRS Paraguaçu a menor (3,53 ng μg^{-1}). Há possibilidade de seleção de genótipos com diferentes concentrações de ricina, que poderão ser utilizados dependendo do interesse dos programas de melhoramento.

Termos para indexação: *Ricinus communis*, endosperma, banco de germoplasma, proteína da semente.

Castor bean (*Ricinus communis* L.) has high oil content in seeds (46–55%) and is an important alternative in the production of biodiesel. Its oil is the only commercial source of ricinoleic acid (12-hydroxy oleic acid). This fatty acid gives the highest viscosity and stability among vegetable oils currently used in the composition of paints, varnishes, lubricants, plastics, and, mainly, cosmetics (Ogunniyi, 2006).

After oil extraction, the remaining processed material can be used as fertilizer to recover depleted soils or as animal feed, due to its high nitrogen content (Godoy et al., 2009). However, the use of these industrial subproducts is limited by the high concentration of ricin, a toxic protein. Ricin penetrates the cell and prevents the production of other proteins, leading to cell death. This happens because the B chain binds to galactose on the eukaryotic cell surface, penetrating the cell and being transported from one cell to another (Sphyris et al., 1995). Once inside the cell, the catalytic action of the A chain enzymatically inactivates the 60S subunit of the ribosomes by depurination of a specific adenine residue in 28S RNA, inactivating protein synthesis. This inactivation is so efficient that a single molecule of this protein is sufficient to kill a cell (Audi et al., 2005). The ricin gene does not contain introns, and there is evidence that it is a member of a multigene family. It is estimated that six copies of the gene encoding ricin are present in the castor bean genome (Halling et al., 1985).

Quantification of ricin in castor bean cultivars may help plant breeding programs and crop management. A study carried out in the castor bean germplasm collection in the United States reported ricin/RCA concentrations of 1.9 to 16 mg g^{-1}, allowing the selection of accessions according to the breeder interest (Pinkerton et al., 1999). Auld et al. (2003), while studying a castor bean population, found ricin and RCA concentrations ranging from 0.10 to 5.60 mg of ricin per gram of seed.

Several methods, varying in accuracy, facility of use, and time required, allow the detection and quantification of ricin (Garber et al., 2005; Lubelli et al., 2006). The detection of proteins by immunoassays has been widely used in clinical diagnostics and in all areas of biological testing. The enzyme-linked immunosorbent assay (ELISA) can identify a protein in a population of other proteins, using crude or semipurified preparations and enzymatic reagents. It is highly sensitive, easy to perform, rapid, and able to generate reproducible results.

The objective of this work was to evaluate ricin concentration in castor bean seeds of 20 accessions from a Brazilian germplasm bank, using ELISA analysis, to assist in the development of strategies for future breeding programs.

Twenty accessions of castor bean from the Banco de Germoplasma de Mamoneira of the Embrapa Algodão, at Campina Grande, PB, Brazil: BRA 3000, BRA 10723B, BRA 10863B, BRA 10391B, BRA 2551, BRA 10341A, BRA 4987, BRA 5916, BRA 6548, BRA 3271, BRA 5908, BRA 3361, BRA 3182, BRA 5894, IAC 2028, IAC 80, IAC Guarani, BRS Energia, BRS Paraguaçu, and BRS Nordestina. Castor bean genotypes were evaluated in a completely randomized block design, with five replicates, and each replicate consisted of two seeds. The means of the genotypes were grouped by the Scott-Knott (1974) test, at 5% probability.

Protein extraction was done by mixing 0.2 g of seed powder with 600 µL sample buffer – 50 mmol L^{-1} of NaH_2PO_4; 20 mmol L^{-1} of NaCl; 2 mmol L^{-1} of PMSF; 10 mmol L^{-1} of DTT, pH 7.0 –, for 30 min at 4ºC. The mixture was centrifuged (18,500 *g*) for 40 min at 4°C, and the supernatant was collected. Total protein was quantified using the quick start Bradford protein assay (Bio-Rad Laboratories Inc., Hercules, CA, USA).

ELISA analysis was carried out according to Baldoni et al. (2010). Basically, 96-well polystyrene plates were coated with 3 µg of total protein (diluted in PBS) and incubated for 4 hours at 37°C. The plates were washed three times with PBS and blocked with 200 µL per well of block solution [PBS, Tween (0.05%), and 2% defatted powdered milk] for 16 hours at 4°C. Then, the plates were washed three times with block solution and incubated with specific goat anti-RTA antiserum rcG-20, (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) diluted in block solution (1:2,000) for 2 hours at 37°C. The rcG-20 antiserum is an affinity purified goat polyclonal antibody raised against a peptide mapping at the N terminus of the ricin precursor of *R. communis* origin. Although Western blot analysis indicated that the proricin precursor and glycoforms of ricin A-chain were recognized specifically, the possibility that the antibody would also detect the homologous RCA A-chain, GenBank accession number P06750, could not be excluded. The plates were washed seven times with block solution and incubated with 50 µL per well of diluted secondary antibody: 1:3,000, rabbit anti-goat IgG conjugated with alkaline phosphatase for 2 hours at 37°C, (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). The plates were washed five times with PBS, and the reaction was carried out using the alkaline phosphatase substrate kit (Bio-Rad Laboratories Inc., Hercules, CA, USA), according to the manufacturer's instructions. Absorbance was measured in a microplate reader (Bio-Rad Laboratories Inc., Hercules, CA, USA) at 405 nm. Experiments were repeated with three biological and three technical replicates. A standard curve was prepared using purified ricin A (Sigma Aldrich Brasil Ltda., São Paulo, SP, Brazil) at concentrations of 0.1, 1, 10, 50, 100, and 500 ng.

There are significant differences among the genotypes for ricin content in seeds, at 5% probability. The coefficient of variation was high (42.73%), indicating that there is variation within the accessions. This result could be explained by the fact that some accessions are from an open-pollinated population, and castor bean has an outcrossing ratio of 19 to 70% (Meinders et al., 1950; Brigham, 1967). In addition, since castor bean has at least six copies of ricin gene in its genome (Halling et al., 1985), differences in the expression in each of these copies, due to mutations and recombinations within a population, could lead to distinct ricin accumulation.

In the analysis of variance, variability of ricin concentration in commercial cultivars and accessions of the Embrapa germplasm bank was significant, indicating that there are significant differences among the genotypes that could be used for selection according to the objective of the breeding program. The varieties IAC 80, IAC 2028, IAC Guarani, BRA 3000, BRA 6548, BRS Nordestina, and BRS Paraguaçu had a small standard deviation when compared to the lines BRA 3271, BRA 5916, BRA 4987, BRA 10341A,

BRA 10391B, BRA 3182, BRA 10723B, BRA 5908, BRA 10863B, BRA 5894, BRA 3361, and BRA 2551. Therefore, the selection of individuals within that population could be done using the single seed descent method.

In the Scott-Knott test for group averages, four distinct groups were identified, with overall average of 15.10 ng μg^{-1} of total protein (Table 1). The first group had the highest concentrations of ricin, with average of 29.44 ng μg^{-1}, while the second group had a mean of 22.02 ng μg^{-1}. The third group, which includes three commercial cultivars – BRS Energia, IAC 80, and IAC 2028–, had the largest number of genotypes and an average of 12.95 ng μg^{-1}. The fourth group had the lowest ricin levels, 6.23 ng μg^{-1} in average. Accession BRA 3271 showed the highest ricin concentration in seeds, i.e., 32.18 ng μg^{-1} of total protein, nine times the content observed for cultivar BRS Paraguaçu, which had the lowest value (3.53 ng μg^{-1}).

After seed oil extraction, the industrial residue of lines and cultivars with low ricin levels can be used for animal feeding. Furthermore, lines with high ricin content can be potentially used to develop high ricin concentration cultivars for therapeutic cancer treatments and chemotherapy (Sandvig & Deurs, 2002; Audi et al., 2005).

There is the possibility of selecting genotypes with different ricin concentrations, which can be used depending on the interest of the breeding programs.

Table 1. Ricin concentration (ng μg^{-1} of total protein) in mature castor bean varieties and accessions from the Banco de Germoplasma de Mamoneira of the Embrapa Algodão.

Group	Genotype	Ricin content[(1)]
1	BRA 3271	32.18±11.21a
	BRA 5916	29.01±11.80a
	BRA 4987	27.14±5.58a
2	BRA 10341A	24.65±6.47b
	BRA 10391B	21.27±8.61b
	BRA 3182	20.14±11.71b
3	BRA 10723B	15.09±6.39c
	BRS Energia	14.61±5.65c
	BRA 5908	13.81±5.84c
	BRA 10863B	13.66±5.69c
	BRA 5894	13.38±6.52c
	BRA 3361	13.36±3.60c
	BRA 2551	12.19±8.52c
	IAC 80	10.23±1.23c
	IAC 2028	10.19±1.16c
4	IAC Guarani	8.80±1.91d
	BRA 3000	7.11±1.76d
	BRA 6548	6.40±2.25d
	BRS Nordestina	5.33±0.82d
	BRS Paraguaçu	3.53±0.95d

[(1)]Means followed by equal letters, in the columns, do not differ by the Scott-Knott test, at 5% probability.

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