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Barriers to Commercialization of a Castor Cultivar with Reduced Concentration of Ricin*

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Ricin is a protein toxin found only in mature castor (*Ricinus communis* L., Euphorbiaceae) seed that enzymatically destroys the ribosomes of eukaryotes. The presence of ricin in the high protein meal of castor has historically reduced its value as an animal feed. Since ricin has the potential to be used as a chemical warfare and bioterrorism agent, the production and processing of castor has undergone increased scrutiny by law enforcement and homeland security agents since the terrorist attacks of Sept. 11, 2001. The production of castor cultivars with reduced levels of ricin would improve the economics of castor oil production; reduce the potential for accidental poisoning; and eliminate the potential of ricin being used by terrorist.

INTRODUCTION

Castor seed contain approximately 50% oil which is composed of 80% to 90% ricinoleic acid (12-hydroxyl-cis-9-octadecenoic acid) (Robbelen et al. 1989). This unique hydroxy fatty acid is used in a number of processes to create unique chemicals and polymers. Annual world production of castor has exceeded one million tonnes (t). In 2003, exports from India were estimated at 185,000 t of oil and oil derivatives at 30,000 t (de Guzman 2004). The volatility of castor oil prices and variability in production from India has made the international market for castor oil very unstable.

Castor seeds also contain two toxins called ricin and *Ricinus communis* agglutinin (RCA_{120}) (Hartley and Lord 2004). Ricin is a ribosome inactivating protein that is manufactured in the endosperm. Ricin has both an A and a B chain which are approximately 30-kDa in size that are linked together by a disulfide bond (Olsnes and Pihl 1973). RCA_{120} is composed of two A-chains and two B-chains linked together by disulfide bonds. Even though RCA_{120} is very similar in both amino acid sequence and structure to ricin, it is much less toxic (Hartley and Lord 2004).

Since 1994, Texas Tech University has been developing castor with reduced levels of ricin. The dwarf-internode cultivar, 'Hale' developed by Texas A&M University in 1970 (Brigham 1970) was crossed with two Plant Introductions from the Soviet Union PI 258368 and PI 257654 which had been previously selected for reduced levels of ricin (Moshkin 1986). In subsequent segregating generations, individual plants were selected for dwarf-internode growth habit and reduced levels of ricin and RCA_{120} using a radial immunodiffusion (RID) assay (Pinkerton et al. 1999; Auld et al. 2001, 2003). In 2003, 12 F_8 lines where intercrossed to develop a synthetic population adapted to mechanical harvest. In 2004 and 2005, this population was screened for semi-dwarf-internode growth habit and lack of shattering. An experimental cultivar derived from this selection process is currently being tested in comparison with the parental cultivar 'Hale' prior to commercial release. In this study, a RID assay and an enzyme-linked immunosorbent assay (ELISA) were used to quantify ricin and RCA_{120} concentration in seed randomly selected from elite populations.

METHODOLOGY

The RID assay used to provide the estimates of ricin and RCA_{120} in the castor seeds was based on ricin specific antibodies. Agarose gel was uniformly heated, mixed with an antibody specific to ricin and then poured onto a plate to solidify (Mancini et al. 1965; Pinkerton 1997). After approximately 30 min, wells are punched into the gel and the plug removed. Experimental samples were pipetted into the individual wells and as the antigen diffused into the gel it bound to the antibody creating a circular (radial) pattern. Staining with Coomassie Brilliant Blue and destaining created a dark blue ring which can be measured with a caliper (Fig. 1). Utilizing the radius obtained from known concentrations of ricin a standard curve was derived for each experiment to allow an estimate of the ricin and RCA_{120} concentration.

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ELISA was used to quantify toxin concentration by binding to a specific antibody (Pathak et al. 1997). In our study, a modified Sandwich /Capture ELISA was utilized in which the labeled antibody to ricin was added to a reaction well that had a specific seed sample containing various concentrations of ricin and RCA_{120} (Vector Laboratories 2004; Lowery 2005). Next a horseradish peroxidase that had been conjugated to a secondary antibody was added to the reaction solution. In the presence of tertamethyl-benzidine, this reaction produces a color change that can be quantified at 450 nm on an ELISA plate reader to provide an estimate the concentration of ricin and RCA_{120}.

Seed samples were assayed from two breeding populations to determine if the concentration of ricin and RCA_{120} to compare the utility of both the RID and ELISA as a breeding tool. One population had been selected for low levels of toxins, short plant height, and the high seed yield necessary for commercial oilseed production. The second population had been selected only for the bright coloration needed when castor is grown as an ornamental. Nine plants (seven oilseed and two ornamental selections) that had been previously selected for low toxin concentrations were grown in two isolated, intercross blocks at Lubbock, Texas in 2004. After harvest, 20 seeds randomly selected from the ornamental population and 30 seed from the oilseed population had total toxin concentration estimated using a modified ELISA technique (Lowery 2005). The oilseed samples had duplicate analyses and the ornamental samples had triplicate analyses.

RESULTS AND DISCUSSION

Two accessions were initially selected from the ornamental population based on RID analyses and accounted for only 1% of the initial population. The 7 accessions selected from the oilseed population made up 5% of the initial population and had less than 3.8 mg/g of ricin and RCA_{120} using a RID analyses (Lowery 2005). The oilseed assay had a standard deviation of 0.16 mg/g of ricin and RCA_{120} (Fig. 2). Earlier work had shown that RID analyses to estimate total ricin and RCA_{120} had been highly repeatable and successful in selecting lines with reduced levels of toxins (Pinkerton et al. 1999; Auld et al. 2003).

The 2004 ELISA assay of 20 seeds randomly selected from the ornamental castor population and 30 seeds selected from the oilseed castor seeds had standard deviations of 3.56 and 8.04 mg/g of ricin and RCA_{120}, respectively (Fig. 3, 4). The standard deviations of the ELISA was from 22 (ornamental population) to 50 (oilseed population) times larger than the 2003 oilseed assay conducted using a RID assay. This high level of variation made selection of individual plants very difficult. In addition, none of the seed in either population had estimated total toxin concentrations lower than 2.8 mg/g. Ricin and RCA_{120} are detectable with an ELISA

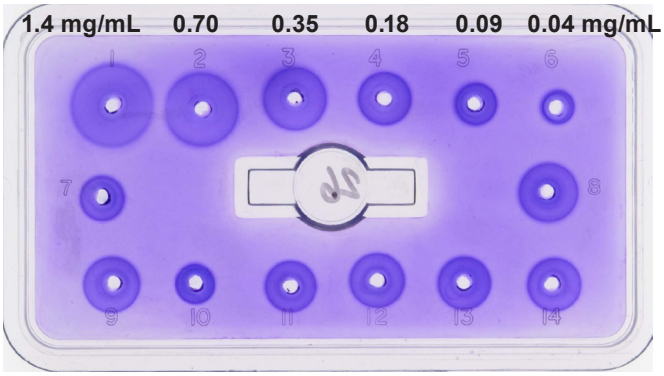

Fig. 1. Radial immunodiffusion (RID) assay results using a graduated series of concentrations of purified ricin in the top row and extracts from castor seeds in the remaining wells.

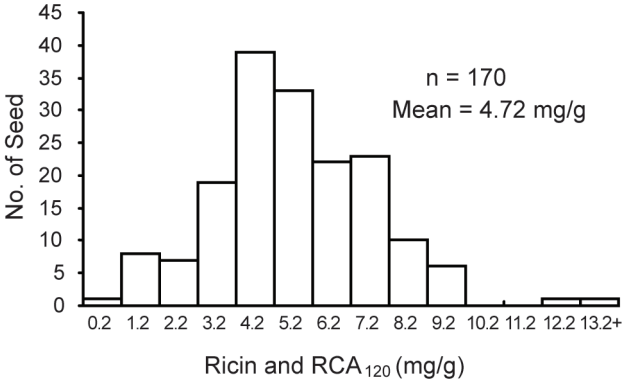

Fig. 2. Distribution of ricin and RCA120 concentration as estimated by Radial Immunodiffusion assay for 170 individual seeds sampled from the 2003 oilseed castor population.

assay at 100,000× dilutions but the assay is sensitive to even minute changes in protocol or the environmental conditions in which it is conducted (Pathak et al. 1997; Lowery 2005).

A similar high level of variation was detected in all 3 of the populations screened using replicated ELISA analyses (Table 1). In 2004, both populations had higher estimated concentrations of ricin and RCA when assayed by ELISA (Fig. 4, 5). The mean for the 2004 ornamental population was 7.92 mg/g of ricin and RCA_{120} with individual values ranging from 2.9 to 25.1 mg/g of ricin and RCA_{120}. The mean for the oilseed population was 17.7 mg/g of ricin and RCA_{120} with individual estimates ranging from 2.8 to 22.9 mg/g of ricin and RCA_{120}. The coefficients of variation for both populations were very high for a laboratory analyses. In addition, the quantitative estimates of total toxins derived by RID and ELISA assays would not have been strongly correlated.

Based on our problems with the ELISA assay we have developed a selection protocol that will use RID analyses for initial selections and confirm final selection with a mammalian cell toxicity assay. We are currently working to adapt a simple and repeatable toxicity assay that is sensitive to ricin and RCA_{120} with our colleagues at the Texas Tech University Health Science Center.

Despite the growing demand for castor oil in both historical industrial oil markets and as a biodiesel feedstock, domestic production of this new cultivar of castor with reduced levels of toxins has been delayed. It will be important to demonstrate a significant reduction in toxins in the meal residue remaining after oil extraction for oilseed processors to address the potential concerns of state and federal agencies with responsibilities for Homeland Security. Hopefully, this new cultivar will reduce or eliminate the potential of extracting ricin from castor meal for use as a weapon of terror.

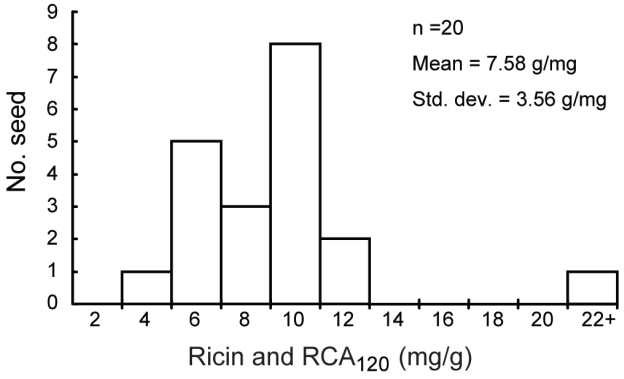

Fig. 3. Estimated ricin and RCA_{120} concentration of 20 individual seeds sampled from the 2004 ornamental castor population assayed by ELISA.

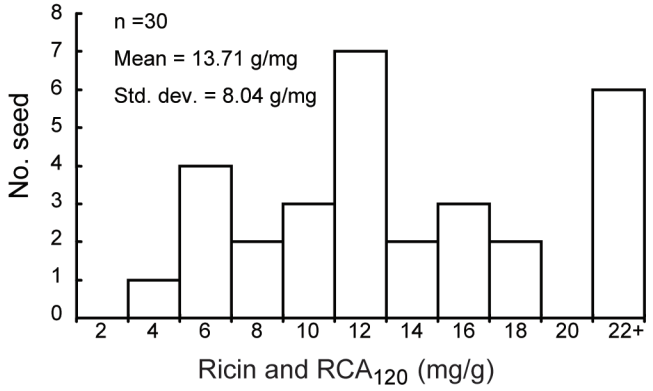

Fig. 4. Estimated ricin and RCA_{120} concentration of 30 individual seeds sampled from the 2004 oilseed castor population assayed by ELISA.

Table 1. Analyses of Variance of three castor populations in which ricin and RCA concentration were assayed by ELISA.

	2003 Oilseed		2004 Oilseed		2004 Ornamental	
Source	df	F Value	df	F Value	df	F Value
Genotypes	9	5.69 **	29	2.34 *	19	2.61 **
Replications	3	2.70 ns	1	0.48 ns	2	2.41 ns
Error	27		22		38	
CV	44.5%		39.8%		52.9%	

*, **, ns denote significant, highly significant and non-significant at the 0.05 level of probability.

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