Lipoxygenase-1 Activity of Soybean Genotypes Grown in Argentina

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Abstract: The lipoxygenase-1 (LOX-1) activity of 19 soybean genotypes was quantified in two consecutive years. The LOX-1 activity produced by any cultivar was essentially the same in both, 1995 and 1996 crop years. The lowest values of LOX-1 activity were found in NK 555 cultivar whereas Asgrow 5409 cultivar had the highest values.

Introduction

Lipoxygenase (LOX) was first reported in soybeans almost 67 years ago [1]. LOX catalyzes the hydroperoxidation of polyunsaturated fatty acids containing a cis, cis-1, 4-pentadiene system, but structures other than fatty acids are known to be oxidised [2, 3]. The cleavage of fatty hydroperoxides into short-chain aldehydes and alcohols has been studied, suggesting that lipoxygenase could be used as a versatile biocatalyst [4].

Normal soybean seeds contain three lipoxygenase isozymes, named LOX-1, LOX-2 and LOX-3 which differ in substrate specificity, optimum pH for catalytic activity, isoelectric point and thermal stability [5, 6]. LOX-1, an enzyme with a pH optimum of 9 to 10, represents a large class of other less-studied LOX of this type. For the biocatalytic production of a natural aroma compound, lipoxygenase is needed on a large scale and the alkaline isozyme (LOX-1) seems to be the most suitable for this purpose [7]. The objectives of this work were to screen and compare the soybean LOX-1 activity in some genotypes cultivated in Argentine in two consecutive years.

Results and Discussion

The biosynthesis of LOX isozymes in soybean is under genetic control [8, 9]. Furthermore, weather conditions have been found to play a considerable role in influencing the activities of the LOX isozymes [7]. In the present work, the differences in activity among the different cultivars from the same year were larger than those between the generations (crop years) of a cultivar. The LOX-1 activity produced by any cultivar was essentially the same in both, 1995 and 1996 crop years (Table 1). These results are not in agreement with the data obtained by Márczy *et al.* [7] suggesting that, in the samples

studied, genetic is stronger than environmental influences.

Table 1. Lipoxygenase-1 activity (ΔOD.mg prot^{-1}.min^{-1}) in 19 soybean cultivars during 1995 and 1996 crop years. Mean values ± standard deviations (n = 3).

Cultivar	Crop year	
	1995	1996
NK 555	7.44 ± 0.14^{a}	7.81 ± 0.16^{a}
Forrest	8.53 ± 0.13^{b}	8.98 ± 0.17^{b}
NK 641	8.75 ± 0.15^{bc}	8.96 ± 0.16^{b}
Tancacha	8.85 ± 0.10^{bcd}	8.66 ± 0.14^{c}
Copetona 53	9.02 ± 0.15^{cd}	9.22 ± 0.19^{b}
Prata	9.24 ± 0.14^{d}	9.51 ± 0.11^{d}
Asgrow 5308	9.83 ± 0.30^{e}	9.96 ± 0.24^{e}
Federada Casilda	10.0 ± 0.20^{ef}	10.8 ± 0.14^{f}
RA 587	10.3 ± 0.10^{f}	10.1 ± 0.13^{e}
Federada 1 INTA	10.9 ± 0.10^{g}	12.3 ± 0.12^{g}
Granera 73	11.4 ± 0.12^{h}	11.2 ± 0.16^{h}
Asgrow 6404	11.5 ± 0.15^{h}	11.6 ± 0.11^{i}
Tacuarí	12.2 ± 0.20^{i}	12.7 ± 0.18^{ij}
Montera 74	12.8 ± 0.10^{j}	13.2 ± 0.12^{k}
RA 702	13.1 ± 0.15^{j}	12.5 ± 0.15^{j}
Torcaza 63	13.1 ± 0.10^{j}	13.3 ± 0.13^{k}
Charata 76	13.9 ± 0.15^{k}	13.4 ± 0.12^{k}
Torcacita 58	14.5 ± 0.20^{m}	14.8 ± 0.16^{m}
Asgrow 5409	16.0 ± 0.20^{n}	16.5 ± 0.23^{n}

^{a}Mean values within each column followed by the same letter do not differ statistically P=0.05.

Statistically significant variations were found among genotypes in both, 1995 and 1996 crop years. A total of 13 groups with different enzymatic activity were observed. In 1995, the LOX-1 activity ranged from 7.44 (NK 555) to 16.0 (Asgrow 5409) ΔOD.mg prot^{-1}.min^{-1}; whereas in 1996 it varied between 7.81 (NK 555) and 15.5 (Asgrow 5409) ΔOD.mg prot^{-1}.min^{-1}. In general, mean values from 1996 were higher than those from 1995, with exception of Tancacha, RA 587, Granera 73, RA 702 and Charata 76 cultivars.

In the last decade, many attempts to improve the flavours of soybean products have centered around the genetic elimination of LOX from the seeds [8-10]. More recently some works [4,6] focus on the potential of LOX for the efficient production of useful compounds. Hence, cultivars with high LOX activity, such as Torcacita 58 and Asgrow 5409, could be used as a source of the Lox-1 isozyme.

Experimental

Plant material

Nineteen soybean genotypes were chosen. The experiment was conducted at the Estación Experimental Agropecuaria (EEA-INTA) of Manfredi, Córdoba, Argentina. Seeds were harvested, in the crop years 1995 and 1996, by hand at maturity when seed moisture was reduced to 10% or less. One hundred seeds (taken randomly from each seed sample) were powdered by grinding and soybean flour of each cultivar was extracted according to Pignata *et al.* [11].

Lipoxygenase assay

The method of Axelrod *et al.* [5] was followed with a slight modification. The activity of LOX-1 isozyme was determined via the increase in absorbance at 234 nm after addition of linoleic acid in 0.1M phosphate buffer (pH 9.0). Lipoxygenase-1 activity was expressed as an optical density increase per mg protein^{-1} per minute^{-1} (ΔOD.mg prot^{-1}.min^{-1}).

Protein content

Protein determinations were performed by the method of Kalckar [12].

Statistical analysis

Lipoxygenase-1 determinations were conducted in triplicate. Statistical differences among genotypes from each crop year were estimated from ANOVA test at the 5% level (P=0.05). Whenever ANOVA indicated significant difference, a pairwise comparison of means by least significant difference (LSD) was carried out.

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References and Notes

1. André, E. *C.R. Acad. Sci. Paris* **1932**, *194*, 645.
2. Pignata, M.L. *Tesis*, Facultad de Ciencias Exactas, Físicas y Naturales, UNC, 1992.
3. Piazza, G.J.; Nuñez, A. *J. Am. Oil Chem. Soc.* **1995**, *72*, 463.
4. Gardner, H.W. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1347.
5. Axelrod, B.; Cheesebrough, T.M.; Lackso, S. *Methods Enzymol.* **1981**, *71*, 441.
6. Siedow, J.N. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1991**, *42*, 145.
7. Márczy, J.S.; Simon, M.L.; Mózsik, L.; Szajáni, B. *J. Agric. Food Chem.* **1995**, *43*, 313.
8. Hildebrand, D.F.; Hymowitz, T. *J. Am. Oil Chem. Soc.* **1981**, *58*, 583.
9. Kitamura, K. *J. Agric. Biol. Chem.* **1984**, *48*, 2339.
10. Kitamura, K.; Davies, C.S.; Kaizuma, N.; Nielsen, N.C. *Crop Sci.* **1983**, *23*, 924.
11. Pignata, M.L.; Acosta, A.T.; Guzmán, C.A. *An. Asoc. Quim. Argent.* **1984**, *72*, 155.
12. Kalckar, H.M. *J. Biol. Chem.* **1947**, *167*, 461.