PHOTOSYNTHESIS AND LEAF LIPOXYGENASE ACTIVITY IN SOYBEAN GENOTYPES LACKING SEED LIPOXYGENASE ISOZYMES[1]

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ABSTRACT- Plants from eight soybean genotypes, including a triple null line (TNL) without seed lipoxygenase isozymes, were grown in a greenhouse to evaluate the effect of genetic elimination of seed lipoxygenases (LOX) on carbon assimilation and leaf LOX activity. Specific leaf matter, carbohydrate and chlorophyll concentration were assessed in plants from the TNL whose floral buds had been continuously removed. There was no difference among genotypes on rates of photosynthesis. Leaf LOX activity, however, was greater in the genotype with the three seed LOX isozymes than in the TNL. The removal of floral buds in the TNL increased leaf LOX activity, as well as, leaf carbohydrate and chlorophyll concentration as compared to control plants, allowed to develop seeds; the contrary was true for the rates of photosynthesis.

Additional index terms: enzyme, carbohydrates, chlorophyll.

FOTOSSÍNTESE E ATIVIDADE DE LIPOXIGENASES NAS FOLHAS DE GENÓTIPOS DE SOJA SEM LIPOXIGENASES NAS SEMENTES

RESUMO- Oito genótipos de soja, incluindo um genótipo triplo nulo, sem LOX nas sementes, foram cultivados em casa de vegetação para avaliar o efeito da eliminação genética das LOX das sementes na fotossíntese e na atividade das lipoxigenases das folhas. A massa foliar específica e a concentração de carboidratos e clorofila foram determinados em plantas do genótipo triplo nulo (TNL) cujas flores tinham sido removidas continuamente. Não houve diferenças entre os genótipos nas taxas de fotossíntese; a atividade das LOX das folhas, entretanto, foi maior no genótipo com os três tipos de LOX nas sementes que no genótipo triplo nulo. As plantas do genótipo TNL mantidas sem flores apresentaram maior atividade das LOX das folhas e maior concentração de carboidratos e clorofila que aquelas que frutificaram normalmente, o contrário observou-se para as taxas de fotossíntese.

Termos adicionais para indexação: enzimas, carboidratos, clorofila.

INTRODUCTION

Lipoxygenases (linoleate:oxygen oxidoreductase, EC 1.13.11.12) are dioxygenases that catalyze the hydroperoxidation of polyunsaturated fatty acids containing *cis-cis* pentadienes moieties. In higher plants, both linoleic and linolenic acids are substrate of LOX. In soybeans (*Glycine max* (L.) Merr.), these fatty acids are present in several plant tissues, especially in the seeds (Siedow, 1991). Seeds of soybeans contain at least three different kinds of lipoxygenase isozymes, called LOX-1 (L-1), LOX-2 (L-2) and LOX-3 (L-3) (Siedow, 1991) which appear to be different from those found in the leaves (Grayburn et al., 1991). Lipoxygenases in soybean seeds promote the peroxidation of polyunsaturated fatty acids which contributes to the formation of undesirable flavor in soybean products.

At the present, a precise physiological function of LOX has not been entirely established, although the enzyme has been involved in growth and development, senescence, and also in plant responses to infection with pathogens (Kato et al., 1992). Further, the study of LOX is receiving renewed interest since they have been involved in jasmonic acid synthesis (Siedow, 1991). Jasmonic acid markedly increases the expression of specific genes, some of which are wound responsive (Staswick, 1992). Depodding of soybean plants may increase

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the levels of leaf carbohydrate and therefore, the rates of photosynthesis may be reduced (Mondal et al., 1978). Whereas removal of pods showed no effects on specific leaf LOX activity in soybean (Tranbarger et al., 1991).

Some genotypes lacking seed lipoxygenases have been obtained at the Universidade Federal de Viçosa, becoming necessary to investigate the effects of removing seed LOX on leaf LOX activity. In this work, leaf LOX activity and photosynthesis in soybean genotypes lacking seed lipoxygenases were investigated. Further, in a soybean seed lipoxygenase null line, photosynthesis, leaf LOX activity, levels of leaf carbohydrates and chlorophyll content were studied in plants whose floral buds had been removed.

MATERIALS AND METHODS

Experiment 1 - Eight soybean genotypes were included in this study: The commercial cv Cristalina with seed LOX, and seven genotypes from the Universidade de Federal de Viçosa germplasm collection, designed -L1, null for L-1; -L2, null for L-2; -L3, null for L-3; -L1L2, null for L-1 and L-2; -L1L3, null for L-1 and L-3; -L2L3 null for L-2 and L-3, and a line lacking the three seed LOX (-L1L2L3), hereinafter referred to as 'triple null line' (TNL). The plants were grown in a greenhouse under a 16 h photoperiod and about 25/19^{o}C day/night temperatures, in 4 L pots containing fertilized potting compost. They were watered as necessary with tap water to prevent water stress.

Enzyme activity was measured in leaves collected from plants at the V3 growth stage (Fehr et al., 1971). Middle leaflets of the second upper fully expanded trifoliate leaves were collected at the midmorning, weighed, immediately frozen in liquid nitrogen, and stored at -80^{o}C until processing (Grimes et al., 1992). The tissue was ground to a powder in liquid nitrogen with chilled mortar and pestle. Following grinding, the total soluble protein was extracted in bidistilled water, 2 mL:1 g of tissue (Grayburn et al., 1991). The homogenate was centrifuged at 12,500 g for 15 min at 4^{o}C and the supernatant, referred to as crude extract, was assayed for LOX activity, at 23^{o}C, based on the spectrophotometric methods of Axelrod et al. (1981). The standard assay mixture (1.03 mL) consisted of 1 mL of sodium phosphate buffer (50 mM, pH 7.0); 5 μL of crude extract and 25 μL of a substrate emulsion (10 mM linoleic acid emulsified in 0.36% Tween-20). The protein content in the sample was determined (Tombs et al., 1959), and then the substrate was added to initiate the enzyme reaction. The formation of hydroperoxides was calculated by using 25,000 $M^{-1}cm^{-1}$ as the extinction coefficient. One unit of LOX activity, one katal, was defined as the amount of enzyme forming 1 mol of hydroperoxide per min.

Photosynthesis was measured at several plant growth stages. At V3 in the TNL and 'Cristalina'; at V12 in genotypes -L1, -L2, -L3, -L1L3, -L2L3, and at R5 in genotypes -L1L2 and in the TNL. It was measured in the middle leaflet of the second upper fully expanded trifoliate leaf between 9:00 and 14:00 under clear sky at a photon flux density of 1000 $\mu mol.m^{-2}.s^{-1}$ or greater on a portable ADC-LCA2/15001 infrared gas analyzer. Other photosynthesis measurements were also taken at irradiance lower than 1000 $\mu mol.m^{-2}.s^{-1}$, between 15:00 and 18:00, in the TNL and 'Cristalina' plants at the V3 growth stage.

Experiment 2 - Plants from the TNL were cultivated as described above. At the beginning of flowering, they were randomly selected for floral bud removal (hereinafter referred to as the desinked plants), while control plants were allowed to flower and develop pods and seeds. In desinked plants all the floral buds were removed twice weekly throughout plant development.

The rates of photosynthesis were measured at the R5 growth stages between 9:00 and 14:00 h under clear sky, as described above. It was used the middle leaflet of the fifth trifoliate leaf beginning at the first trifoliate leaf from the bottom of the plant in both desinked and control plants. In control plants, it was verified that the leaf used for photosynthesis measurements content at least one pod at its node. Further, photosynthesis, enzyme activity, specific leaf matter, chlorophyll and carbohydrates contents were assessed at the same trifoliate leaf. Enzyme activity and photosynthesis were measured in the middle leaflet, whereas specific leaf matter, chlorophyll and carbohydrates were determined in lateral leaflets.

The enzyme was assessed as described above, with some modifications, based on previous leaf LOX activity assays (Marenco, R.A.; Lopes, N.F. & Moreira, A.A. - unpublished data). The standard mixture assay consisted of 1 mL of phosphate buffer (50mM, pH 6.6); 7.5 μL of substrate emulsion (10-mM linoleic acid emulsified in 0.36% Tween 20) and 2.0 μL of crude extract. Leaf LOX activity was assayed at the V3, R4 and R5 growth stages in the TNL and only at the V3, V7 and V12 growth stages in the cv Cristalina.

Chlorophyll was determined by the method of Lichtenthaler (1987) at the V3, R5 and R6 growth stages in the TNL and at the V3 stage in the cv Cristalina. Specific leaf weight (SLW) was calculated as SLW = LW/LA, where LW is the leaf dry matter, determined after drying at 70^{o}C for 48 h and LA is the leaf area, measured on a Licor (LI-3000) leaf

area meter. Total soluble carbohydrates, extracted from fresh leaflets in boiling 80% ethanol, were purified with chloroform. The starch in the ethanol soluble residue was extracted in 6.9% perchloric acid, being the precipitate removed by centrifugation (15 min, 5000 g). When necessary the samples were stored at -20^{o}C until processing. Carbohydrates were assessed spectrophotometrically at 620 nm, after their reaction with anthrone reagent (0.1% p/v, anthrone in sulfuric acid). Glucose was used as the standard. The starch content in the sample was calculated by multiplying the observed value by 0.9.

RESULTS AND DISCUSSION

Experiment 1 - Leaf LOX activity was lower in the genotypes: -L1, -L2L3, and TNL than in 'Cristalina' (Fig. 1). The higher leaf LOX activity observed in 'Cristalina' as compared to the TNL plants suggests an effect of seed LOX genetic elimination on leaf LOX activity, which may be important for soybean production since lipoxygenases have been involved in plant response to pathogen attack (Kato et al., 1992; Rickauer, et al., 1992) and wounding (Siedow, 1991). No effect on photosynthesis rates was observed as a consequence of genetic removal of seed LOX (data not shown). Even though there was effect of seed genetic elimination on leaf LOX activity, all the genotypes tested, including the TNL, grew and developed well, which suggests that no essential physiological role appear to be associated to seed LOX isozymes. Siedow (1991) suggested that a compensatory regulation take place in mutant lines to increase the expression of one cotyledon LOX isozyme when another is missing. Therefore, if any leaf LOX isozyme is missing as a consequence of seed LOX genetic elimination, a regulatory mechanism could be activated so that growth and development may occur normally, as observed in this experiment.

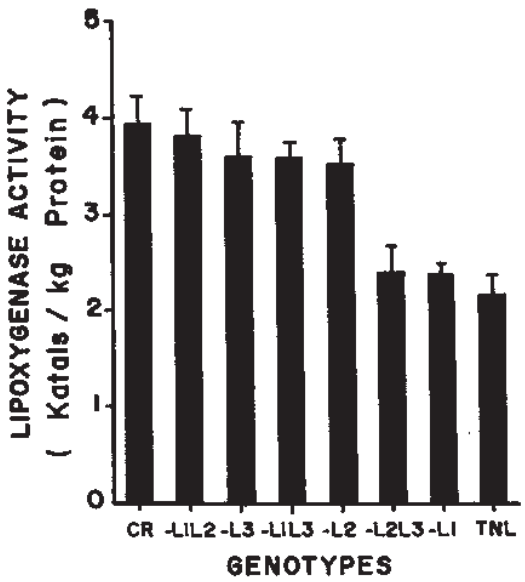

FIGURE 1- Leaf lipoxygenase (LOX) activity in soybean plants at the V3 growth stage. The genotypes included were: 'Cristalina'(CR), with seed LOX, and seven genotypes lacking seed LOX, designed, -L1, null for LOX-1; -L2, null for LOX-2; -L3, null for LOX-3; -L1L2, null for LOX-1 and LOX-2; -L1L3, null for LOX-1 and LOX-3; -L2L3 null for LOX-2 and LOX-3, and -L1L2L3 (TNL), null for LOX-1, LOX-2 and LOX-3. (n=4; bar, SE).

TABLE 1- Rates of photosynthesis ($\mu mol.m^{-2}.s^{-1}$) in 'Cristalina' and triple null line (TNL) plants at the V3 and R5 growth stage.

Genotype	Growth stage	Leaf measured	Treatment	CO_2 assimilation1
'Cristalina'	V3	2nd	-	10.39 a
TNL	V3	2nd	-	9.99 a
TNL	R5	5th	control	2.86 b
TNL	-	5th	desinked	1.29 c

1/ Means followed by the same letter are not significantly different at P = 0.01 by the Duncan test.

Experiment 2 - There was no difference in chlorophyll and carbohydrates concentration between 'Cristalina' and the TNL at the V3 growth stage. In addition, the same rates of photosynthesis were observed at either high or low levels of irradiance (data not shown), which verified the results obtained in experiment 1.

Desinked TNL plants showed lower rates of photosynthesis than control plants (Table 1), which was probably due to the high levels of carbohydrates, especially starch, accumulated in desinked plants (Table 2). Inhibition of photosynthesis by sink removal is consistent with previous results (Mondal et al., 1978; Wittenbach, 1983). The activity of LOX was higher in TNL desinked than in control plants at both R4 and R5 growth stage (Table 3). A greater leaf LOX activity in desinked plant may be probably a plant defense mechanism since jasmonic acid, a secondary product of lipoxygenase action upon linolenic acid (Siedow, 1991), may induce protease inhibitor in distant untreated leaves (Staswick, 1992).

TABLE 2- Total soluble carbohydrate (TSC, $g.m^{-2}$), starch ($g.m^{-2}$), specific leaf matter (SLW, $g.m^{-2}$), and chlorophyll (Chl, $mg.m^{-2}$) in leaf of control and desinked triple null line plants, without seed lipoxygenases, at two growth stage (GS).

Treatment	GS	TSC	Starch	SLW	Chl-a	Chl-b[1]
control	R5	1.36 b	9.46 b	33.0 b	88.0 bc	73.0 ab
desinked	-	1.88 a	18.19 a	53.0 a	111.0 b	81.0 ab
control	R6	1.61 a	3.23 c	29.0 b	64.0 c	54.0 b
desinked	-	1.65 a	16.96 a	49.0 a	158.0 a	105.0 a

1/ Means followed by the same letter are not significantly different at P = 0.05 by the Duncan test.

TABLE 3- Leaf lipoxygenase activity ($Katals.kg^{-1}$ protein) in desinked and control triple null line plants, without seed lipoxygenases, at both R4 and R5 growth stages.

Treatment	Growth stage	Leaf measured	LOX activity
Control	R4	5th	0.070 a
Desinked	-	5th	0.118 b
Control	R5	5th	0.087a
Desinked	-	5th	0.125 b

1/ Means followed by the same letter are not significantly different at P = 0.01 by the Duncan test.

The removal of floral buds increased the specific leaf weight and chlorophyll content, mainly at the R6 growth stage (Table 2). Higher values of specific leaf weight in desinked plants did verify a continued photosynthate production following floral bud removal as suggested by Wittenbach (1983). From this study it may be concluded that genetic elimination of seed LOX appears to have no effect on the rates of photosynthesis. On the other hand, even though TNL plants grew and developed seeds normally, they showed lower level of leaf LOX activity, which may be important from a physiological point of view, since lipoxygenases catalyze the synthesis of jasmonic acid.

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