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Ethylene and Lipoxygenase In Relation to Afterripening of Dormant NC-13 Peanut Seeds[1,2]

D. L. Ketring* and H. E. Pattee[3]

ABSTRACT

Following harvest, peanuts are usually subjected to a period of storage. During storage biochemical changes are known to occur. The objectives of this study were to determine the changes and relationship in ethylene production, germination, and lipoxygenase (LG) activity during cold storage of dormant NC-13 peanut seeds. Two seed lots (SL) were used: one grown in Oklahoma (SL80) and the other grown in N. Carolina (SL81). SL80 and SL81 were stored at 2 to 5 C for 193 and 242 days, respectively. Samples were taken at about 28-day intervals for determination of germination, ethylene production, and LG activity. Seeds of two and three maturities were tested for SL80 and SL81, respectively. As afterripening of stored seeds proceeded, ethylene production gradually increased, with the maximum at 48 hours of germination. Germination showed a concomitant gradual increase. Lipoxygenase activity of both seedlots was less for mature than for immature seeds and showed a sharp increase during storage at 2 to 5 C, particularly for immature seeds. After heat-treatment to break dormancy of sublots from SL81, there was a progessive increase in ethylene production and germination, but most notably for mature seeds. In contrast to ethylene production and germination, after heat-treatment LG activity declined. Linear correlation coefficient (r) values between ethylene production and germination were highly significant for mature seeds from SL80 at 48 and 72 hours of germination, but only at 72 hours for immature seeds. For SL81 as for SL80, significant positive correlations were found between ethylene production and germination. However, correlations between LG activity and the other variables were not significant except for mature seeds from SL81. Significant positive correlations for both ethylene production and germination with LG activity also existed for these seeds. But after heat-treatment these correlations no longer occurred. The data indicate that the metabolic processes related to ethylene production and germination are occurring simultaneously with those of LG activity. The possibility that metabolites from LG activity serve as substrates for ethylene production can not be precluded.

Key Words: *Arachis hypogaea* L., groundnut, germination, storage, enzyme, metabolism.

Peanuts following harvest are subjected to storage. It is estimated that 75-80% of the edible trade portion passes through cold storage for periods which may extend beyond one year, but average three to four months (17). During storage of both edible-trade and seed peanuts, biochemical changes are known to occur. In edible trade peanuts these changes involve free amino acids (14), free sugars (15), flavor (2,16), and volatile production capacity (12). Quantitative changes in volatile production capacity have been suggested to be a reflection of metabolic processes which affect the flavor quality of raw peanuts and their roasted flavor potential (12,18). Pentane, acetaldehyde, and methanol accounted for 80 to 98% of the volatiles, and the first two of these components accounted for 55 to 75%. These three components arise as products of lipoxygenase (LG) and pectin methyl esterase activities. A need exists to establish that LG activity in peanuts is changing with storage time as volatile production capacity changes.

Dormancy in seed peanuts and its changes with storage is an important factor in commercial peanut production. Dormancy is absent or lasts only a few weeks in spanish and valencia types, but may last up to four months or longer in virginia-type peanut seeds. The virginia-type cultivar, NC-13, has a high degree of dormancy and is widely used as the seed source in dormancy related studies. Using this cultivar, Ketring and Morgan (5,6,7) have elucidated the role of ethylene in dormancy regulation in peanuts. Peanut dormancy is apparently caused by endogenous metabolic barriers that are probably enzymatic in nature and prevent protein synthesis and/or the degradation and transport of food reserves to the embryo (8). Although the evidence favors methionine as the major precursor of ethylene in most plant tissues, and this may be the case for peanut seeds during early hours of germination (4), there are indications that other metabolites may serve as substrates for ethylene biosynthesis. The $^{13}C/^{12}C$ ratio in ethylene from avocado fruit (a fatty tissue) was found to change as ripening progressed, indicating that ethylene may be derived from amino acids at early ripening and then from fatty acids at later stages of ripening (10). Dormant peanut seeds which are high in fat content also progress through stages of afterripening. Also, it has been suggested that linolenic acid may play some essen-

[1]Cooperative Investigations of the Southern Region, Southern Plains and South-Atlantic Areas, USDA-ARS, the Oklahoma Agric. Exp. Stn., Stillwater, OK 74078, and the North Carolina Agric. Res. Service, Raleigh, NC 27695.

[2]Mention of a trademark name or a proprietary product does not imply its approval to the exclusion of other products that also may be suitable.

[3]Plant Physiologist, USDA-ARS, Plant Science Research Laboratory and Dept. of Agronomy, Oklahoma State Univ., Stillwater, OK 74076; Research Chemist, USDA-ARS, North Carolina State Univ., Box 7625, Raleigh, NC 27695.

tial secondary role in ethylene biosynthesis in apple tissue (11). Unsaturated fatty acids such as linoleic and linolenic can serve as substrates for LG activity in peanut seeds. An enzyme system that requires linoleic acid and lipoxygenase to produce ethylene has recently been described (1).

The timing of flavor changes, volatile production capacity, and dormancy loss in virginia-type peanut suggests a possible interaction between these events. The objectives of this study were to determine the effect of storage time on extractable LG activity, germination, ethylene production, and their relation.

Materials and Methods

Peanut (*Arachis hypogaea* L.) cv. NC-13 seeds were used in this study. This genotype has a high degree of fresh seed dormancy (5,6). For the first experiment, plots were planted at Fort Cobb, OK, 15 May 1980 and pods harvested 18 September 1980. For the second experiment plots were planted at Clayton, NC, 19 May 1981 and pods harvested 8 October 1981. Plots were irrigated as necessary. After harvesting, curing, and during shelling, two and three seed maturity classes were selected for testing from the 1980 (SL80) and 1981 seed lot (SL81), respectively. The classes were descriptively similar to 9 and 11 for SL80 and 7, 9, and 11 for SL81 according to the classification of Pattee *et al.* (11). These are designated MC-7, MC-9, and MC-11 in the tables and figures where applicable. Seeds were stored at 2 to 5 C and about 60% RH until samples were taken for testing or sublots were heat-treated to break dormancy. Cold temperature retains dormancy of these seeds over an extended period (6 to 8 months), while at room temperature dormancy declines more rapidly (30 to 60 days), and a heat-treatment (40 C for 14 days) will break dormancy (5,19). Testing began at 25 days after harvest and at 28-day intervals thereafter for SL80. The last seeds (193 days) for testing SL80 were kept at room temperature for the 28 days prior to the final test. This was done to determine seed response to a dormancy-breaking treatment since the seeds remained mostly dormant (germination was only 35% at 165 days after harvest, throughout the experiment. Testing began at 56 and 67 days after harvest for LG activity and ethylene production and germination, respectively, for SL81. The main portion of the seed lot was kept at 2 to 5 C. Samples designated (A) were removed for testing at 28-day intervals until the end of the experiment. In addition, three sublots were taken at 95 (B Samples), 123 (C Samples), and 151 (D Samples) days after harvest for heat-treatment (40 C for 14 days) to break dormancy. The sublots were then returned to cold storage. Seeds from the B, C, and D sublots were tested 14 days after heat-treatment and then at 28-day intervals until the end of the experiment.

Ethylene production and germination procedures were as previously reported (5), except that the gas chromatograph was a Beckman Model GC-4 and the oven was operated at 65 C. Also, in these tests 10 seeds per replication from the bulk lot were germinated at 29±1 C in 250 mL flasks fitted with two sheets of 9-cm diameter Whatman No. 5 filter paper. Distilled water (15 mL) was added to each flask for seed imbibition and to maintain moist conditions throughout the 72-hour germination period.

Lipoxygenase extracts were prepared in the following manner. Ten peanut seeds were ground in a Sorvall Omni-Mixer using 25 mL 0.05M Tris buffer pH 7.5 for 1 min at a #7 setting. The slurry was quantitatively transferred to a centrifuge tube using 5 mL Tris buffer as wash solution and then centrifuged for 20 min at 27,000XG and 4 C. The fat layer was removed and the supernatant transferred to a 50 mL volumetric flask. The pellet was resuspended in 15 mL Tris buffer, ground, and centrifuged as described. The resulting fat layer was removed and the supernatant added to the first extraction supernatant and brought to 50 mL volume with 0.05 M Tris buffer pH 7.5. The complete extraction procedure was conducted at approximately 4 C and the LG extracts kept in an ice bath during assay.

Enzyme activity was measured polarographically at 25 C in a reaction vessel fitted with a Clark oxygen electrode. The vessel contained 3.3 μmoles of linoleic acid in 0.1 mL Borate-NaOH buffer, 50μL enzyme extract, and 1.35 mL of 0.1M phosphate buffer for a total volume of 1.5 mL and final pH of 6.2. Activity was calculated from the slope of the polarographic trace on assumption that the initial O_2 concentration was 260 nmoles/mL (3).

Enzyme assays were replicated 3 times, and each replicate assayed twice. Mean values of LG activity (CU/min/g seed) plotted in the figures are for six measurements. Duplicate and triplicate samples of 10 seeds for each maturity class were used for testing germination and ethylene production of SL80 and SL81, respectively. Data were analyzed by standard analysis of variance procedures, and differences between mean values were determined by Duncan's multiple range test or least significant differences (LSD).

Results and Discussion

Seeds of NC-13 have been shown to retain their natural inherent dormancy (< 10% germination) for up to 12 weeks after harvest, with an initial germination test at 6 weeks after harvest (6). Thus, although SL80 and SL81 were initially tested at 25 and 67 days after harvest (DAH), respectively, the seeds would still show their inherent dormancy from date of harvest.

Seeds from SL80 were apparently more dormant than those of SL81, as indicated by lower ethylene production (Fig. 1A and 1B) and germination (Fig. 2A and 2B) by 1980 seeds than by 1981 seeds during early sampling dates (1-4) following continuous storage at 2 to 5 C. There was no significant difference in ethylene production between seed maturity classes in either year. The data for maturities were then combined and are presented for each sampling time (Lines labeled 1-7 in Fig. 1A and 1B). Ethylene production of SL80 seeds at early sampling times (1-4, Fig. 1A) was less at 48 hr than at later sampling times (5-7, Fig. 1A). Germination showed a concomitant increase with ethylene production at sampling time 5 (137 DAH, Fig. 2A) for mature seeds. But germination was still only about 30%. Ethylene production was lowest at the first sampling date for SL81 seeds (A samples, Fig. 1B), but showed a distinct maximum at 48 hr, which was significantly higher for the remaining samples. Germination also was the least at the first sampling time (67 DAH, Fig. 2B) but was twice that of the SL80 seeds through 81 DAH (Fig. 2A). The earlier attainment of ethylene maxima at 48 hr and higher percentage germination indicates SL81 seeds were less dormant than SL80 seeds. Both maximum ethylene production at 48 hours and the natural increase (as opposed to treatments to break dormancy) in germination during storage agree with previous results (5,6). The difference in initial sampling time for SL80 and SL81 has no significant effect on the data trends. It also has been previously shown that the afterripening process in peanut is quiescent for 60-90 days after harvest (12,14,15). Thus, observations taken in the first 60-90 days after harvest should still reflect the initial inherent dormancy at harvest. The earlier higher rates of ethylene production and increase in germination for SL81 compared to SL80 might suggest that intensity of the dormancy factor from year to year partially controls the initiation of the afterripening process.

Mean LG activity changed only slightly for mature and immature seeds, respectively, until 137 DAH for SL80 (Fig. 2C). Mature seeds (MC-11) had less LG activity than immature seeds (MC-9). Between 137 and 165 DAH, LG activity rose sharply for immature seeds, but increased only slightly for mature seeds (Fig. 2C). The LG activity remained high for immature seeds but declined for mature seeds at 193 DAH. As with SL80,

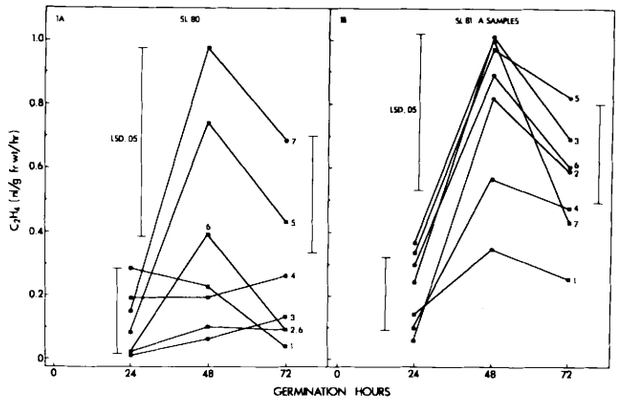

Fig. 1. Time sequence of ethylene production by SL80 (1A) and SL81 (1B) NC-13 seeds. Numbers adjacent to the curves indicate the sampling dates (1 through 7, 25 to 193, and 67 to 235 days after harvest for 1980 and 1981 seeds, respectively). Vertical bars indicate the LSD, 0.05 at each hour of measurement.

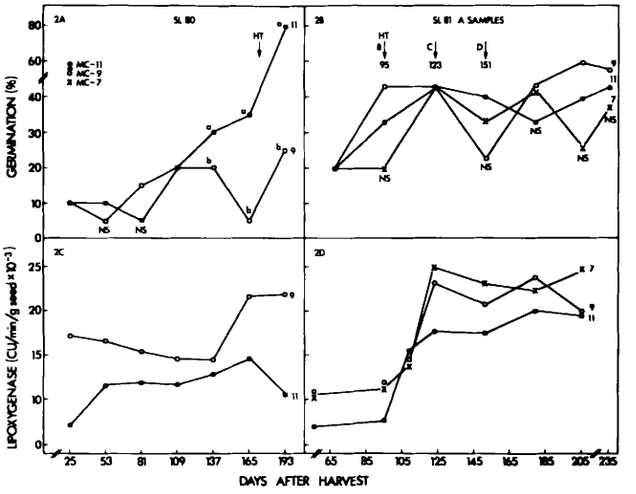

Fig. 2. Germination of SL80 (2A) and SL81 (2B) and mean values of lipoxygenase activity of SL80 (2C) and SL81 (2D) NC-13 seeds. Small letters (a,b) adjacent to germination data points indicate significant differences between seed maturity classes (MC-7, X; MC-9, 0; MC-11, ●). B, C, D indicate samples heat treated (HT) at the time shown by arrow. NS-not significant.

LG activity of mature seeds from SL81 was less than that of immature seeds (Fig. 2D). Also, there was a sharp rise in LG activity, but this occurred earlier (109 to 123 DAH) for seeds from SL81 (Fig. 2D) than for seeds from SL80 (Fig. 2C). The SL81 seeds which had a lower degree of dormancy had an earlier rise in LG activity. The LG pattern for SL81 is in agreement with the volatile profile pattern observed across storage time by Pattee *et al.* (12), which also showed a sharp increase to a maximum near 120 days after harvest and thus supports the concept that an increase in extractable lipoxygenase activity was partially responsible for the volatile profile change. The earlier discussed potential interaction between dormancy intensity and the afterripening process may also be suppressing the lipoxygenase activity profile for the SL80 seeds. The SL80 seeds were more dormant than the SL81 seeds.

At 165 days after harvest of SL80, germination of mature seeds (MC-11) was only 35%, and immature seeds (MC-9) germinated only about 20% (Fig. 2A). At this time the remaining sample (No. 7) of SL80 seeds was placed at room temperature to reduce the degree of dormancy. The final test of these seeds at 193 days after harvest showed increased ethylene production (Fig. 1A) and germination (Fig. 2A), as expected, particularly for the mature seeds. However, LG activity decreased for mature seeds but remained near the same high level for immature seeds (Fig. 2C).

To further assess the effect of breaking dormancy on the seeds, sublots of SL81 were heat-treated. The day of heat-treatment and subsample designations (B,C,D) are shown in Fig. 2B. There was a progressive increase in germination (Fig. 3A and 3B) and ethylene production (Fig. 4) with time after heat-treatment, and immature seeds showed the least germination. This agrees with the results for SL80 seeds. Previously it was found that exogenous ethylene was not effective in stimulating germination of intact immature peanut seeds (7). Apparently the increase in endogenous ethylene production after heat-treatment also was mostly ineffective in increasing germination of immature seeds (MC-7) used in these studies. The seeds heat-treated at 151 days after harvest (D samples, data not shown) responded similarly to the B and C samples (Fig. 3A, B, C, and D).

In contrast to ethylene production and germination after heat treatment, the LG activity showed a general decline with days after harvest, particularly for mature seeds (Fig. 3C and 3D). Also, the mature seeds had the

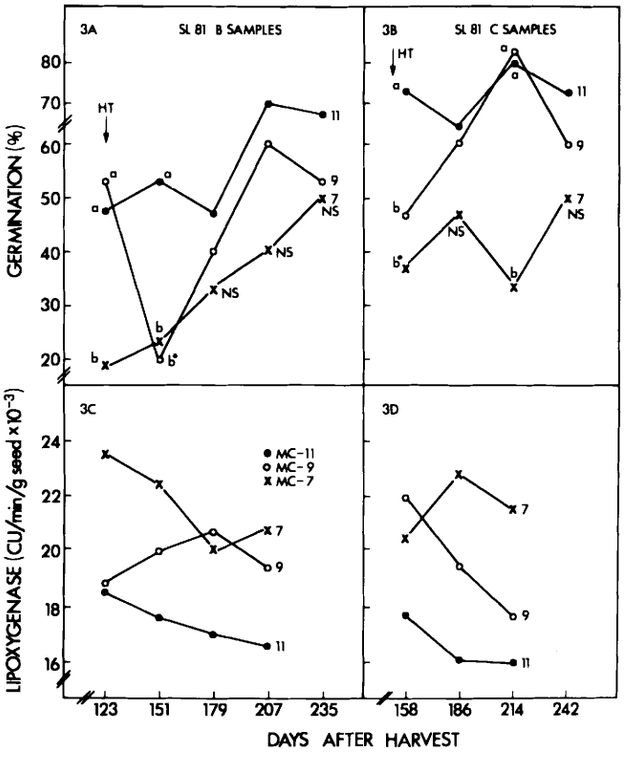

Fig. 3. Germination (3A and 3B) and mean values of lipoxygenase activity (3C and 3D) of heat-treated sublots B and C of SL81 NC-13 seeds. Other letters and numerical designations are the same as for Fig. 2 Significant differences were at the 0.05 level of probability, unless indicated by an asterisk*, which were at the 0.10 level.

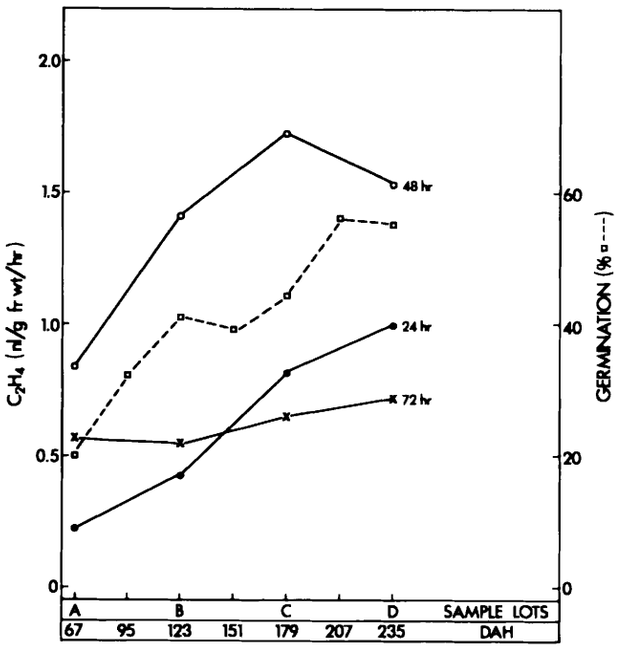

Fig. 4. Ethylene production means (all observations for a given sample lot e.g. A, B, C, D) at 24, 48, and 72 hours and germination means (all observations for a given day after harvest (DAH)) at 72 hours for SL81 NC-13 seeds. A samples, continuous cold storage; B, C, & D samples, heat-treated at 95, 123, and 151 DAH, respectively.

Table 1. Values of the correlation coefficient (r) between dormant NC-13 seeds ethylene production, germination, and lipoxygenase activity from SL80.

	% Germination Immature†	% Germination Mature	Lipoxygenase Immature	Lipoxygenase Mature
C_2H_4 24 hr	0.33	0.09	-0.08	-0.74*
C_2H_4 48 hr	0.62	0.90***	0.47	0.03
C_2H_4 72 hr	0.86**	0.86**	0.25	-0.12
ΔC_2H_4 24-48 hr	0.52	0.87**	0.49	0.25
ΔC_2H_4 48-72 hr	0.08	0.63	0.61	0.21
Lipoxygenase Immature	-0.12	-	-	-
Lipoxygenase Mature	-	0.01	-	-

† Immature, MC-9; mature, MC-11.
***,**,* Significant at the 0.01, 0.05, and 0.1 levels of probability, respectively.

least and the immature seeds the most enzyme activity. This agrees with response of the seeds from SL80.

In order to determine relationships between ethylene production, germination, and LG activity, linear correlation coefficient (r) values were calculated between these variables. Germination and ethylene production of mature seeds (MC-11) from SL80 were highly correlated at 48 and 72 hours of germination (Table 1). The change in ethylene production between 24 and 48 hours of germination was also correlated with mature seed germination. These parameters were only correlated at 72 hours for immature seeds (MC-9), which have slower radicle emergence than mature seeds. These correlations are in agreement with the observation that visual, radicle emergence usually occurs at 48 hours for most seeds of this virginia-type cultivar. Lipoxygenase activity was negatively correlated with ethylene production at 24 hours for mature seeds (Table 1). This might be expected since ethylene production was generally increasing throughout the storage period (Fig. 1) while LG activity of mature seeds remained nearly constant (Fig. 2C). Correlations betweens LG activity and other parameters were low and not significant (Table 1).

For SL81 as for SL80, significant correlations were found between ethylene production and germination (Table 2). This occurred at 48 hours for all maturity classes from the A samples, and maturities 7 and 11 from the B samples. The r values for the C samples were not significant or were negative. This was due to the large amounts of ethylene being produced relative to the increase in germination that occurred. A threshold level of ethylene production sufficient to stimulate dormant seeds to germinate was previously postulated (5,6), and these data support this view. However, large amounts of ethylene production have been associated with vigor of nondormant spanish-type, but not virginia-type seeds (9). Lipoxygenase activity of seeds from SL81 was significantly correlated with both germination and ethylene production of mature seeds from A samples which were afterripened continuously in cold storage (Table 2). However, after heat-treatment (B and C samples) this correlation no longer occurred. This was due to the opposite effects of heat-treatment on LG activity and on ethylene production and germination. Enzyme activity declined after heat-treatment (Fig. 3C and 3D), while ethylene production and germination increased (Fig. 4). Seeds from both SL80 and SL81 showed a sharp increase in LG activity after a period of cold storage. Seeds in mature classes (11) showed less LG activity than seeds in more immature classes (7 and 9), and LG activity of mature seeds from SL81 was correlated with both ethylene production and germination. The data indicate that the metabolic processes related to ethylene production and germination are occurring simultaneously with those of LG activity, but apparently are only indirectly associated with them. However, the possibility that metabolites from LG activity serve as substrates for ethylene production can not be precluded.

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Table 2. Values of the correlation coefficient (r) between dormant NC-13 seeds ethylene production, germination, and lipoxygenase activity from SL81.

	Ethylene Production				Lipoxygenase Activity		
	24 hr	48 hr	72 hr	24-48 hr†	7	9	11
A Samples							
GERMINATION							
Seed Maturity							
7‡	0.647	0.678*	0.613	0.512	0.506	-	-
9	0.694*	0.817**	0.552	0.664	-	0.409	-
11	0.415	0.741*	0.438	0.721*	-	-	0.755**
	df=5				df=5		
LIPOXYGENASE ACTIVITY							
Seed Maturity							
7	0.562	0.289	0.225	0.059	-	-	-
9	0.503	0.272	0.320	0.070	-	-	-
11	0.732*	0.527	0.346	0.270	-	-	-
	df=5						
B Samples							
GERMINATION							
Seed Maturity							
7	0.893**	0.884**	0.718	0.249	0.842	-	-
9	0.706	0.737	0.134	0.281	-	-0.588	-
11	0.888**	0.806*	0.148	0.083	-	-	-0.617
	df=3				df=2		
LIPOXYGENASE ACTIVITY							
Seed Maturity							
7	-0.526	-0.266	-0.774	0.879	-	-	-
9	-0.100	-0.353	-0.814	-0.536	-	-	-
11	-0.731	-0.477	-0.533	0.973**	-	-	-
	df=2						
C Samples							
GERMINATION							
Seed Maturity							
7	0.283	0.459	0.021	0.132	0.710	-	-
9	0.185	0.361	0.071	0.181	-	-0.824	-
11	-0.912*	-0.624	-0.999***	0.918	-	-	0.897
	df=2				df=1		
LIPOXYGENASE ACTIVITY							
Seed Maturity							
7	0.977	0.921	0.992*	-0.974	-	-	-
9	-0.714	-0.830	-0.648	0.343	-	-	-
11	-0.929	-0.981	-0.891	0.678	-	-	-
	df=1						

† Change in ethylene production between 24 and 48 hours of germination.

‡ Seed maturity stages.

***, **, *Significant at the 0.01, 0.05, and 0.1 level of probability, respectively.

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