Ageing in Tomato Reduces the Capacity of Seeds to Produce Ethylene, While Priming Increases Ethylene Evolution During Germination

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Introduction

During germination, there is often a rise in ethylene production following imbibition that peaks just after radicle emergence (Matilla, 2000). Increased ethylene production during the early stages of germination has been documented for numerous species (Lalonde and Saini, 1992; Petruzzelli et al., 1993; Khan, 1994). Although this increased ethylene production appears to be common among diverse species, studies using ethylene inhibitors or ethylene-insensitive mutants demonstrate that non-dormant seeds do not generally require ethylene production to complete radicle emergence (Lalonde and Saini, 1992; Hua and Meyerowitz, 1998).

The ability of a seed lot to produce ethylene following imbibition has consistently been associated with seed viability and is negatively correlated with seed ageing (Samimy and Taylor, 1983; Gorecki et al., 1991; Khan, 1994; Chojnowski et al., 1997). In all cases, aged seed lots produced ethylene later after imbibition and produced less ethylene overall than non-aged seed lots. One possible mechanism reducing ethylene production in aged seeds is a reduction in 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase activity or synthesis. In general, seeds exposed to saturating concentrations of exogenous ACC amplify subsequent ethylene production, but the magnitude of this increased production is considerably greater in non-aged vs. aged seeds (Khan, 1994), suggesting reduced ACC-oxidase activity in the latter. Siriwitayawan (2002) showed reduced ACC-oxidase mRNA abundance in aged tomato seeds.
Seed priming reduces the time to radicle protrusion and often ameliorates the deleterious effects of seed ageing on germination (McDonald, 2000). The mechanisms for improved germination in primed seeds are not completely understood, but maintaining seeds in the lag phase of germination is thought to promote cellular repair and initiate metabolic processes required for germination. There are several studies that show an increased ability for primed seeds to produce ethylene during germination (Fu et al., 1988; Chojnowski et al., 1997; Cantliffe et al., 2000; Habdas et al., 2000). However, it is not clear whether ethylene production is integral to obtaining a priming effect in seeds or whether it is simply the result of higher vigour displayed by primed seeds.

The objective of the present study was to compare ethylene production in a tomato seed lot after moderate ageing. A seed lot aged for 18 months was chosen, because it showed reduced seed vigour but maintained high standard germination. Therefore, differences in ethylene production would be due to vigour loss without being confounded with reduced viability. In addition, the relationship between seed priming and ethylene production in aged and non-aged seeds was compared with the intent of using ethylene-insensitive mutants in tomato to study whether there is a causal relationship between ethylene and the priming response in seeds.

Materials and Methods

Tomato seeds (*Lycopersicon esculentum* L. Mill. cv. Moneymaker) were originally obtained from the C.M. Rick Tomato Genetics Resource Center (TGRC) (University of California at Davis) and subsequently grown at the University of Kentucky Horticultural Research Farm. Seeds were collected from mature fruits and placed in sealed glass bottles and stored at 4°C or room temperature (~23°C) for 18 months. Standard germination was in Petri dishes on moist blotters incubated at alternate 8 h at 30°C in light (20 μmol/s/m²) and 16 h at 30°C in dark (AOSA, 1992). Four replications of 50 seeds were tested for standard germination.

Seeds were osmotically primed by placing 2000 seeds in 500 ml of an aerated 3% KNO₃ solution for 7 days at 20°C in the dark and subsequently dried at room temperature for 24 h to a moisture content of 8.2 ± 1.1% (fresh weight basis).

In separate Petri dishes, seeds were imbibed on water or ACC (5 mM) for various times (12, 24, 36, 48 and 60 h). Petri dishes were placed in germination conditions as previously described. Ethylene evolution was quantified by moving 50 seeds from the pool of ACC-treated seeds to dry 25 ml Erlenmeyer flasks. Flasks were sealed with serum stoppers. After 3 h of incubation, a syringe was used to withdraw a 1 ml gas sample for ethylene evaluation. A Buck Scientific gas chromatograph with flame ionization detector (155°C) and alumina column (125°C) with a nitrogen flow rate of 1 ml/min was used to determine ethylene concentration using a standard curve of dilutions of pure ethylene in air.

Four replications of 50 seeds were frozen in liquid nitrogen and placed
in a mortar and pestle and ground in 2 ml of 80% ethanol. The slurry was transferred into a test tube and incubated at 70°C for 30 min. Following centrifugation, the supernatant was removed and evaporated to dryness in vacuo. Subsequently, chloroform (1 ml) plus water (1 ml) was used to resuspend and separate recovered ACC into the aqueous phase, which was assayed for ACC-derived ethylene production according to methods from McKeon et al. (1982) and Lizada and Yang (1979). Internal standards for ACC indicated extraction efficiency for the assay of 88.0 ± 1.9%.

**Results and Discussion**

Tomato seeds aged for 18 months showed only an 8% reduction in final germination percentage (data not shown) but a significant reduction in seed vigour as indicated by radicle emergence after 60 h (Fig. 48.1) and accelerated ageing test (data not shown). Tomato seeds germinating on water evolved less ethylene compared with seeds imbibed in the presence of 5 mM ACC (Fig. 48.1). ACC content in seeds imbibed on 5 mM ACC increased from less than 5 to approximately 500 and 1500 pmol per seed after 12 and

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**Fig. 48.1.** Time to radicle protrusion and ethylene evolution from tomato seeds aged for 18 months with or without priming and imbibed on water (A, C) or 5 mM ACC (B, D). Closed symbols non-aged, open symbols aged, circles non-primed, triangles primed. Values are means ± standard error. Means followed by the same letter were not different using Tukey’s test ($P \leq 0.05$).
24 h, respectively. Aged seeds showed a reduced capacity to evolve ethylene and this was more apparent in ACC-treated seeds (Fig. 48.1). Non-primed aged seeds began to evolve ethylene 24 h later than non-primed non-aged seeds. Khan (1994) has suggested that ethylene produced during germination in the presence of exogenous ACC would be a sensitive biochemical test for seed vigour. Several other studies have come to the same conclusion that ethylene evolution is diagnostic for seed vigour (Samimy and Taylor, 1983; Gorecki et al., 1991; Chojnowski et al., 1997). However, in these studies, accelerated ageing was used to deteriorate seeds, resulting in a significant reduction in viability as well as vigour. Seed vigour begins to be reduced prior to loss in viability. The current study indicates that even between seed lots with comparable viability, reduced vigour results in a reduced capacity to produce ethylene (Fig. 48.1).

Non-primed tomato seeds attained 50% radicle protrusion after 77 h, while primed seeds required only approximately 36 h (Fig. 48.1). Priming was accompanied by a significant increase in ethylene production during radicle protrusion compared with non-primed seeds. At 48 h, primed seeds produced ten times the ethylene of non-primed seeds (Fig. 48.1). Primed aged seeds recovered most of the vigour lost during storage. Primed aged and non-aged seeds had similar radicle emergence percentages after 48 h. However, ethylene production, though enhanced, was significantly lower in primed aged seeds compared with primed non-aged seeds (Fig. 48.1C). Primed seeds imbibed on ACC produced an order of magnitude more ethylene compared with non-primed seeds (Fig. 48.1D). The ethylene production of primed aged and primed non-aged seeds germinated on 5 mM ACC was comparable, only differing at 48 h.

A correlation between priming and ethylene evolution during germination has been shown in a number of species (Esahsi et al., 1990; Chojnowski et al., 1997; Habdas et al., 2000). Priming appears to reduce the time required to initiate synthesis and/or activity of both ACC-synthase and ACC-oxidase. Fu et al. (1988) showed that ACC content and ACC-synthase activity were increased in groundnut (Arachis hypogea L.) seeds by osmotic priming. In the current study, endogenous ACC content in tomato seeds following priming was 4.0 ± 0.9 pmol per seed compared with 0.69 ± 0.08 and 1.1 ± 0.06 pmol per seed in non-primed non-aged and non-primed aged seeds, respectively. Based on the ability to produce ethylene during germination, it appears that endogenous ACC may not increase in primed aged seeds to the same degree as in primed non-aged seeds. Ethylene evolution in primed aged seeds was one-fourth that of primed non-aged seeds after 48 h when both sets of seeds had similar percentages of radicle protrusion (Fig. 48.1). ACC-oxidase activity, as indicated by the ability to convert saturating levels of ACC to ethylene, was greatly increased by priming (Fig. 48.1B). There was less difference between primed aged and primed non-aged seeds when imbibed on ACC relative to on water, but primed non-aged seeds still produced twice the ethylene at 48 h compared with primed aged seeds.

The significance of ethylene production to the priming process has received some attention. Cantliffe et al. (2000), investigating the interaction
between priming and ethylene production in thermoinhibited lettuce (*Lactuca sativa* L.) seeds, determined that ethylene promoted endo-β-mannanase activity, which was partly responsible for inducing germination at elevated temperatures. A second interaction between ethylene production and seed priming may involve ethylene-mediated osmoregulation (Esahsi et al., 1990). Primed cocklebur (*Xanthium pennsylvanicum* Wallr.) as well as ethylene-treated seeds showed increased osmolarity in their cell sap, enabling them to complete germination on mannitol solutions with reduced water potential.

The ethylene-insensitive *Nr* mutation in tomato was used to investigate further the interaction between ethylene and seed priming (Fig. 48.2). The time to radicle protrusion in *Nr* seeds was significantly reduced compared with wild type seeds (*T* 50 for wild type was 75 h, *Nr* was 52 h). This has been shown to be more related to the impact of reduced ethylene perception during seed development, rather than a direct effect of ethylene perception on germination speed (Siriwitayawan, 2002). Seeds from both *Nr* and wild-type plants responded in a similar way to priming by significantly reducing the time to radicle emergence compared with non-primed seeds (Fig. 48.2). This suggests that ethylene perception is not required for seeds to show the benefits of priming. However, there are multiple receptors in tomato for ethylene and it is possible that additional receptors (i.e. *LeETR2* or *LeETR4*) act independently from *Nr* during seed germination to allow ethylene perception. Given this limitation, the maintenance of a seed priming effect in *Nr*

![Fig. 48.2. Time to radicle protrusion from wild type (wt) or never ripe (Nr) tomato seeds before and after priming. Closed symbols wild type, open symbols Nr, circles untreated, triangles primed. Values are means ± standard error. Means followed by the same letter were not different using Tukey's test (P ≤ 0.05).](image-url)
tomato seeds is the best evidence to date to indicate that ethylene perception is not a requirement for reduced time to radicle protrusion in primed seeds.

References


