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Research Note

Ethanol release as an indicator of seed vigour in radish, pepper, watermelon, aubergine, leek and onion seed lots

Eda Burcu Kucukhuseyin[1], Nurcan Memıs[2], Zeynep Gokdas[2], Steven P.C. Groot[3] and Ibrahim Demir[2]*

[1] Agriculture and Forestry Ministry, General Directorate of Plant Production, Ankara, Turkey
[2] Ankara University, Agriculture Faculty, Horticulture Department, Ankara, Turkey
[3] Wageningen University & Research, Plant Science Group, Wageningen, The Netherlands
* Author for correspondence (E-mail demir@agri.ankara.edu.tr)

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Abstract

Ethanol release was tested in relation to seed vigour measured by seedling emergence and controlled deterioration tolerance in radish, pepper, watermelon, aubergine, leek and onion seed lots. Ethanol release, measured with six lots from each species after four and eight hours at 40°C and 30% seed moisture, was highly negatively correlated with controlled deterioration tolerance (45°C, 48 hours, 20% seed moisture) in radish ($r = 0.05$, $P < 0.05$), with seedling emergence in watermelon ($r = 0.99$, $P < 0.01$) and to both traits in pepper ($r = 0.87$ to 0.94, $P < 0.05$), but not with any variable for aubergine and leek. One single case was significant between ethanol release after eight hours and controlled deterioration for onion seeds. Results indicate that there is a relationship between ethanol release and seed vigour, but this appears to be species dependent.

Keywords: controlled deterioration, seedling emergence, seed quality

Experimental and discussion

Seed vigour is the main feature of seed quality that indicates the field emergence potential of a seed lot (Matthews *et al*., 2012). Vigour traits are influenced by seed production, post-harvest treatments and storage. Seed lots that have aged more have lower emergence and shorter longevity than less aged seed lots. Ageing has various effects that reduce seed lot performance. One of these is damage to membranes, including in mitochondria , which impairs aerobic energy metabolism. Loss of mitochondrial integrity due to ageing can result in anaerobic respiration and ethanol release, which has been associated with seed vigour in various crop seeds (Pesis and Ng, 1984; Gorecki *et al*., 1985; Kataki and Taylor,

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1997; Taylor *et al*., 1999; Rutzke *et al*., 2008; Buckley and Buckley, 2009; Buckley, 2013). Ethanol release measured using a modified breath analyser was successfully used as a rapid test to rank the vigour of seed lots of canola (Buckley and Buckley, 2009) and cabbage (Kodde *et al*., 2012). However, ethanol release as an indicator of seed vigour should be investigated in a wider range of species. The objective of the present study was to determine the relationship between ethanol release and seed vigour (controlled deterioration tolerance and seedling emergence percentages) of radish, pepper, watermelon, aubergine, leek and onion seed lots.

Seeds of six lots of each of six species, radish (*Raphanus sativus* L. cv. Fındık), pepper (*Capsicum annuum* L. cv. Carliston), watermelon (*Citrullus lanatus* Matsum and Nakai (Thunb.) cv. Crimson Sweet), aubergine (*Solanum melongena* L. cv. Kemer), leek (*Allium porrum* L. cv. Inegol) and onion (*Allium cepa* L. cv. Mtn 88), were obtained from commercial companies. Seed moisture contents (ISTA, 2020) ranged between 6.5 to 8.2%.

Standard laboratory germination tests (SG) were conducted using four replicates of 50 seeds. Seeds were placed between damp filter paper (Filtrak, Germany). Papers were then placed in plastic bags. Germination tests were carried out at 20°C for radish, onion and leek, and 25°C for watermelon, pepper and aubergine, in the dark. Standard germination was evaluated after 14 days for watermelon, leek, aubergine and pepper, after 12 days for onion and after 10 days for radish.

For ethanol release measurement, seed moisture content (mc) of two replicates of 0.5 g of seeds was increased to 30% by placing seeds on top of wet Whatman filter paper for 6-7.5 hours. Seeds were then hermetically stored at 5°C overnight in aluminium packets to allow moisture to equilibrate within each sample. Then, 0.5 g seeds were placed into 15 ml dark glass vials (816030-2170) for 3-4 seconds and placed at 40°C. Headspace ethanol was measured as $\mu g\ L^{-1}$ after 4 and 8 hours (EtOH-4 and EtOH-8) using a modified Drager 6810 (Drager Safety AG and Co. KGaA, Germany) alcohol breath analyser as described in Kodde *et al*. (2012). Measurements were performed within five seconds to avoid cooling and vials were then put back in the incubator.

For controlled deterioration (CD), seed mc was increased by placing samples of 200 seeds with known mc on wet filter paper in a Petri dish to reach 20% mc. Then, they were sealed in foil packets and placed at 5°C for one day for moisture equilibration. Packets were then placed at 45°C for 48 hours and standard germination tests were conducted on four replicates of 50 seeds as described above.

The seedling emergence (SE) test was determined by sowing four replicates of 50 seeds 30 mm-deep in seedling trays with compost (Plantaflour, Humus–Verkaufs GmbH, Germany). The trays were placed in a growing cabinet where the temperature was maintained at 23 ± 2°C. Light was provided for 16 hours per day (72 $\mu M\ m^{-1}\ second^{-1}$). The experiment was terminated when no additional seedling emergence occurred.

Correlation coefficients (r) for ethanol release after four and eight hours and SG, CD and SE tests and regression coefficients (R^2) were calculated by using SPSS in each species.

SG values ranged between 75 and 95% among the species. All seed lots had higher germination than the commercially required minimum level (75%) (table 1). Germination after the CD test showed wide variation among the species. Germination of onion seed lots after CD was between 50 and 83%, with watermelon between 39 and 86%. While some radish and leek seed lots had germination as low as 0 and 1% after CD, pepper and aubergine had a similar range after CD, between 29-85% and 31-82%, respectively.

There was also wide variation in the seedling emergence range (SE) among the species. The smallest difference between the lowest and the highest quality lot was measured in aubergine, varying over 77-93%, and the largest difference was found for pepper with a 51-91% range. The other species had comparable SE range between the worst and the best seed lots, i.e. 39-76% in leek and 48-84% in watermelon.

The highest ethanol release was observed in radish at both measurement times. The sample from the seed lot with the lowest SG had ethanol release of 2591 µg L^{-1} after four hours and 2782 µg L^{-1} after eight hours (table 1). Onion seeds had the lowest ethanol release: 2-90 µg L^{-1} after four hours and 3-10 µg L^{-1} after eight hours. With the exception of onion, seeds of all other species had increased ethanol release at the second measurement (eight hours).

Table 1. Standard germination (SG), germination after controlled deterioration (CD), seedling emergence (SE), ethanol release after four (EtOH-4 µg L^{-1}) or eight hours (EtOH-8 µg L^{-1}) of six seed lots of each of radish, pepper, watermelon, aubergine, leek and onion. The observed range (minimum and maximum values) is given for each parameter. Correlation coefficient (*r*) values between EtOH-4 and EtOH-8 and SG, CD and SE are given in italics for each species.

Species	EtOH release (µg L^{-1})	SG (%)	CD (%)	SE (%)	
Radish		82 – 95	0 – 74	63 – 91	
	EtOH-4 : 34 – 2591	*0.38*	*0.90**	*0.68*	+
	EtOH-8 : 26-2782	*0.44*	*0.91**	*0.75*	
Pepper		75 – 92	29 – 85	51 – 91	
	EtOH-4 : 174-756	*0.74*	*0.89**	*0.92***	+
	EtOH-8 :184-977	*0.78*	*0.87**	*0.94***	
Watermelon		75 – 97	39 – 86	48 – 84	
	EtOH-4 : 23-968	*0.77*	*0.73*	*0.99***	+
	EtOH-8 : 34-1472	*0.74*	*0.67*	*0.99***	
Aubergine		78 – 90	31 – 82	77 – 93	
	EtOH-4 : 67-224	*0.68*	*0.48*	*0.57*	–
	EtOH-8 : 54-361	*0.51*	*0.64*	*0.67*	
Leek		80 – 91	1 – 72	39 – 76	
	EtOH-4 : 26-643	*0.12*	*0.63*	*0.11*	–
	EtOH-8 : 12-787	*0.16*	*0.66*	*0.13*	
Onion		79 – 93	50 – 83	60 – 89	
	EtOH-4 : 2-90	*0.07*	*0.74*	*0.31*	–
	EtOH-8 : 3-10	*0.79*	*0.92***	*0.87*	

+ more promising, – less promising, Significance *0.05; **0.01

Ethanol measurements were not significantly correlated with SG for any of the species (table 1). The significance ($P < 0.05$) was measured for pepper, radish and watermelon seeds. The highest significant correlation was seen between ethanol release and SE for pepper and watermelon ($r = 0.92$ to 0.99, $P < 0.01$). None of the combinations were significant for aubergine and leek seeds. One single case was significant for onion seeds between CD and EtOH-8. EtOH-4 was significantly related ($P < 0.05$, $R^2 = 0.79$-0.81) with CD for pepper and radish seed lots (figure 1). Measurement after the same length of time was also highly related to SE for pepper ($P < 0.05$, $R^2 = 0.85$) and for watermelon ($P < 0.01$, $R^2 = 0.97$).

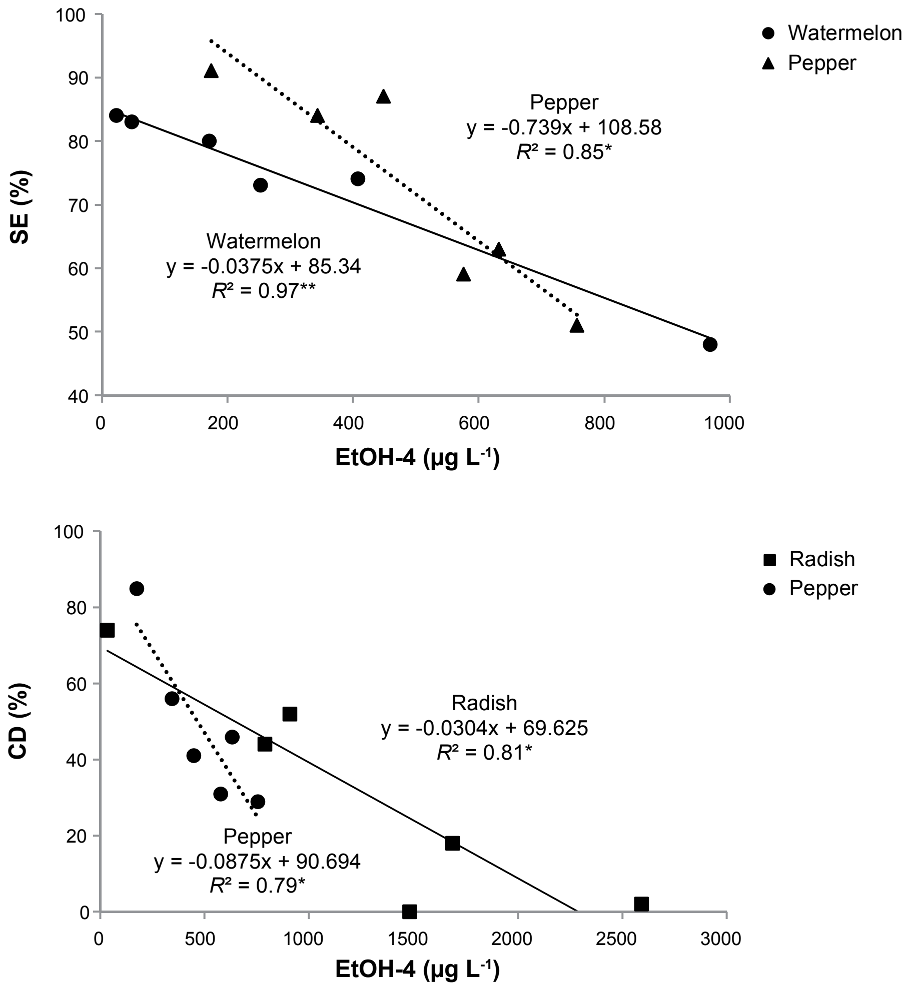

Figure 1. The relationship between ethanol release after four hours, (EtOH-4 / µg L^{-1}) and seedling emergence (SE, %) in watermelon and pepper, controlled deterioration (CD, %) in radish and pepper seed lots. (Significance *= 0.05; **= 0.01).

Fast seedling emergence is an important aspect of crop establishment and transplant production in vegetable seeds. Seed vigour tests are reported to indicate the physiological ageing level of a lot (Matthews *et al*., 2012) and are used to estimate its emergence potential. Ageing that causes differences in vigour of a seed lot is composed of various metabolic events (McDonald, 1999). One of these is membrane degradation, which causes loss of mitochondrial structure and higher release of ethanol upon imbibition (Kataki and Taylor, 1997). We demonstrated that radish, pepper and watermelon seed quality was related to ethanol release (table 1; figure 1). This finding is in agreement with a number of earlier reports (Pesis and Ng, 1984; Gorecki *et al*., 1985; Kataki and Taylor, 1997; Taylor *et al*., 1999; Rutzke *et al*., 2008; Buckley and Buckley, 2009).

However, ethanol measurements appeared to be unsuccessful in predicting emergence and relationship with CD results, at least with the present mc and ageing periods, for aubergine, leek and onion seed lots (table 1). The sensitivity of an ethanol assay is influenced by incubation temperature, seed mc, seed integrity and oxygen availability. Incubating seeds at 40°C instead of moderate temperatures (20-25°C) was more successful (Rutzke *et al*., 2008; Buckley and Huang, 2011; Kodde *et al*., 2012) and this is why we chose 40°C for our study. Our results indicated that 30% seed mc could be used for determining ethanol production from these vegetable crop species. This is in agreement with findings of Rutzke *et al*. (2008) that 30% is an appropriate seed moisture content to see the vigour differences of aged seed lots. Kodde *et al.* (2012) moistened the seeds by adding water at the start of the assay at 40°C. Here, we equilibrated the seeds first for 6-7.5 hours at 20°C, followed by an overnight equilibration at 5°C. It is possible that some mitochondrial repair occurred during this period, which may have influenced subsequent ethanol production. Alternatively, the lack of a significant relationship between ethanol measurements and SE and CD in aubergine, leek and onion seed lots may originate from seed integrity/morphological differences among species. Similarly, Kataki and Taylor (1997) reported that some species like corn, soybean and pea produced larger amounts of ethanol than those of rice and lettuce. Rutzke *et al*. (2008) also reported that crushed cabbage seeds produced less ethanol than intact seeds. Moreover, rapid hydration during germination can cause damage to cell components in seeds with damaged coats in the same seed lot (Oliveira *et al*., 1984), which may affect ethanol release. Low oxygen availability/nitrogen reduced ethanol production in non-aged seeds when seed moisture was low ($\leq$ 0.54 g water/g seeds) at ambient oxygen of $\geq$ 1.22 g water/g seeds elevated ethanol production (Rutzke *et al*., 2008). Ethanol release is also induced by anaerobic environments when seed moisture is high through hypoxic conditions. However, we did not compare aerobic and anaerobic conditions in our work.

In conclusion, the increase in ethanol production from aged seeds in watermelon, pepper and radish seeds supports the hypothesis that ethanol production is correlated with the seed vigour potential of seed lots. However, the lack of such a correlation in aubergine, leek and onion seeds shows that further experiments with adaptation of the assay are required for these species.

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