

**Effect of Vitrification Solution (PVS2) on Viability and Vigour of seeds of *Amaranth*
(*Amaranthus Hybridus*)**

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Abstract

An experiment was conducted on the effects of different exposure periods of plant vitrification solution 2 (PVS2) on viability and vigour of seeds of *Amaranth* (*Amaranthus hybridus*). This was done as a step towards long term cryopreservation of seeds of *Amaranth*. The seeds of *Amaranth*, 100 per treatment, were exposed to PVS2 between 0 and 60 min. Data were taken on in vitro germination percentage of the seeds, percentage of moisture removed from the seeds, shoot and root length of the seedlings and their vigour index calculated. The results showed that PVS2 application did not have an adverse effect on germination percentage of the seeds when the exposure time was not more than 45 min. However, the root and shoot lengths as well as the vigor of the seedling were inhibited to a certain extent by the PVS2 application. PVS2 application dehydrated the moisture of the seeds during the exposure. About 34 to 60.7 % of the moisture was removed from the seeds by the PVS2. This work may pave the way for cryopreservation of seeds of *Amaranth* and similar seeds.

Keywords: *Amaranthus*, seed, cryopreservation, PVS2, desiccation

INTRODUCTION

Vegetables are an integral part of balanced diet and are considered as protective foods as their consumption prevent several diseases (Williams, 1991). Among the indigenous vegetables which are widely consumed across the length and breadth of the tropics is *Amaranth* which belongs to the family *Amaranthaceae*. *Amaranth* locally known as spinach green or ‘Alefu’ in Ghana is one of the most popular leafy vegetables in West Africa especially in the drier savannah areas (Tweneboah, 2000). *Amaranth* is a fast growing crop with high yield potential (30 t / ha for fresh leaves and 4.5 t / ha for dry

leaves) when harvested at four weeks from direct sowing. It is suitable for crop rotation with any other vegetable crops, less susceptible to soil-borne diseases than most other vegetables. The leaves have nitrate and oxalate levels similar to other green leafy vegetables (Tweneboah, 2001). According to Abbey *et al.* (2006) *Amaranth* leaves contain 23.5-27.0 % crude protein.

Amaranth seeds are tiny, glossy, usually black and biconvex (Norman 1992). They are orthodox and are usually conserved at dry places with moisture content of 10-12 %. Even under ideal conditions, orthodox seeds have finite life spans, spanning between years

and centuries, depending on the species (Walters *et al.*, 2005). The life span of seeds in hydrated storage even under the best conditions is variable among species and various attempts have been made to extend it (Berjark and Pammenter, 2008).

Cryopreservation as a long term storage strategy for seed germplasm is needed to assure preservation of plant genetic resources. Cryopreservation in Liquid Nitrogen (LN) (-196 °C) reduces the rate of seed deterioration and thus increases the storage life of the seed. Stanwood and Sowa (1995) reported that Cryopreservation resulted in higher onion seed vigour when compared with storage at -18 °C in refrigerator. Cryopreservation is considered the most appropriate procedure for long-term preservation of genetic resources and reduces genetic variation frequently associated with extended culture of suspensions at physiological normal temperatures (usually about 25 °C) (Towill, 2004). Cryogenic storage provides long term storage at a reasonable cost with low labour input (Trigiano and Gray, 2000).

Storage in LN most often requires dehydration of the tissue with concentrated vitrification solution in order to induce dehydration of the tissue. This is to prevent ice crystal formation at low temperature which will damage the seed. Tissues treated with vitrification solution increase their solute concentration and thus making them viscous thereby inhibiting the coming together of water molecules to form ice (Benson, 2008; Lambardi 2005; Sakai 2000). Exposure to vitrification solution is a necessary step before tissues are exposed to ultra low temperature provided by liquid

nitrogen. Prominent among the vitrification solutions is plant vitrification solution 2 (PVS2) developed by Sakai *et al.* (1990). Over exposure of tissue to PVS2 is detrimental to the tissue. Period of exposure of seeds to vitrification solution that will ensure sufficient dehydration and still allow cell survival after cryopreservation is critical. The objective of this research was to find the suitable exposure period of *Amaranth* seeds to PVS2 that will ensure dehydration and viability. It is anticipated that this research will provide information that will enable successful cryopreservation of *Amaranth* seeds.

MATERIALS AND METHODS

Laboratory experiments were conducted to investigate the effect of PVS2 on germination percentage and vigour of seeds of *Amaranth* as influenced by different PVS2 exposure periods viz., 0, 30, 45 and 60 min. The experiment was carried out at the Plant Tissue Culture laboratory of the University for Development Studies, Nyankpala Campus in the Tolon District of Northern Region of Ghana. It is on latitude 9^o25¹N, longitude 0^o58¹W and at 183 m altitude above sea level.

Murashige and Skoog (MS) media preparation

The *in vitro* culture of seeds takes place on a medium. The most popular medium used in Plant tissue culture is Murashige and Skoog (MS). One litre of MS media was prepared by weighing 4.41g of MS and dissolving in deionised water. Sucrose (20 g) was weighed and added to the solution. Benzylaminopurine (1000 µm) was added to

the solution as growth regulator. The pH of the solution was adjusted to 5.7 ± 1 using 1 N NaOH before 2 g of gelling powder (Agar) was added. The medium was then autoclaved for 40 min at 16 psi and 121°C and poured into 9 cm Petri dishes in a laminar flow hood to avoid contamination.

PVS2 preparation

PVS2 consists of Glycerol (30 %), Ethylene glycol (15 %), Dimethylsulfoxide (15 %) and 0.4 M sucrose in liquid MS. 50 ml of PVS2 was prepared by measuring 15, 7.5 and 7.5 ml of glycerol, ethylene glycol and DMSO respectively into a beaker. 20 ml of liquid MS containing 6.85g of sucrose (0.4 M) was added. The solution was then filter sterilized using $2\ \mu\text{m}$ filter and aliquoted into 7 ml Bijou containers (Sterilin Ltd, Stone, UK) and stored in freezer.

Preparation of deloading solution

Deloading solution is a highly concentrated medium that is used to wash off PVS2 from the seeds. It consists of 1.2 M sucrose prepared in MS medium. 300 ml of the deloading solution was prepared by dissolving 123 g of sucrose in 300 ml MS solution.

Seeds surface sterilization

Seeds of *Amaranth* were surface sterilized to disinfect or eliminate contamination. The seeds were exposed to 1.5 % sodium hypochlorite for 10 min; it was then rinsed three times in sterile distilled water. The seeds were again rinsed in 70 % ethanol for 30 seconds. Finally, the seeds were soaked in 1 % sodium hypochlorite for 15 min, followed by three rinses in distilled sterilized

water. All sterilization processes were carried out in laminar flow hood to avoid contamination from the environment.

Dehydration of seeds with PVS2

After the sterilization the seeds were exposed to PVS2 for different time durations to determine the suitable time exposure to ensure dehydration. The exposure periods to PVS2 were 0, 30, 45 and 60 min in three replicates with 100 seeds per treatment. After dehydration of the seeds the PVS2 was pipetted off and the seeds were kept in deloading solution for 20 min at room temperature to remove PVS2 from the seeds. The seeds were then cultured on solidified MS medium contained in 9 cm Petri dishes. The Petri dishes were arranged in a complete randomized design and were cultured at 25°C under 16/8 hour photoperiod in a growth room.

Data collection and analysis

Data were collected on the moisture content of seeds of *Amaranth* before and after PVS2 treatment. Ten days after culturing, data were taken on the following parameters; shoot and root lengths of the seedlings. The mean number of seeds that germinated by the tenth day was recorded for each treatment and germination percentage was calculated as

$$\frac{\text{Number Of Normal Seedlings}}{\text{Total Number Of Pure Seeds Sown}} \times 100$$

The vigour of the seedlings was measured using the vigour index. Ten days after sowing the seeds on MS medium, the seedlings were uprooted and the gel was carefully washed to prevent damage to the roots. The shoot and root lengths of each seedling were measured

to prevent damage to the roots. The shoot and root lengths of each seedling were measured with a ruler. The average length of roots and shoots per treatment was calculated and the vigour index determined using the formula; Vigour index = (Shoot length + Root length) X % germination (Abdul-Baki and Anderson, 1973). Data collected were subjected to analysis of variance (ANOVA) using the computer statistical package Genstat Discovery edition four. Least Significant Difference (LSD) test at 5% probability was used to determine treatment differences.

RESULTS

Effect of PVS2 exposure period on germination percentage of seeds of *Amaranth*

There were significant differences ($p < 0.05$) in germination percentage. Seeds exposed to PVS2 for 60 min recorded significantly lower percentage germination than the control treatment (Table 1). There was no significant difference in germination percentage among the control, 30 and 45 min PVS2 exposure periods.

Table 1. Percent germination of seeds of *Amaranth* exposed to different periods of PVS2

PVS2 Exposure period (min)	Germination (%)
0	94.7
30	76.0
45	82.3
60	62.0
LSD (0.05)	28.9

Amount of moisture removed from *Amaranth* seeds due to PVS2 application

There was significant difference ($p=0.01$) in the amount of moisture removed from the seeds due to PVS2 application. The amount of moisture removed from seeds exposed to PVS2 increased with increasing exposure time (Table 2). There was no significant difference in amount of dehydration between 45 and 60 min exposure periods.

Table 2. Percent moisture removed from *Amaranth* seeds exposed to different PVS2 duration

PVS2 Exposure period (min)	Moisture content (%)
0	0.0
30	34.0
45	50.0
60	60.7
LSD (0.05)	11.6

Effect of PVS2 exposure on root length of germinating seeds of *Amaranth*

There were significant differences ($p=0.03$) in root growth of germinating seeds due to the application of the PVS2 to the seeds (Figure 1). The PVS2 application inhibited elongation of the roots of germinating seeds. Root length decreased with increasing period of exposure. Exposure of seeds to PVS2 for 30 and 45 min inhibited root growth by 33 % and this was increased to 52.6 % when the exposure period was extended to 60 min.

Effect of PVS2 exposure on shoot growth of germinating *Amaranth* seeds

Longer exposure periods of PVS2 inhibited shoot length elongation of germinating seeds (Figure 2). Exposure of seeds to PVS2 for 30 and 45 min inhibited shoot length by about

38.5 % and this was increased to 42.9 % when the exposure period was extended to 60 min.

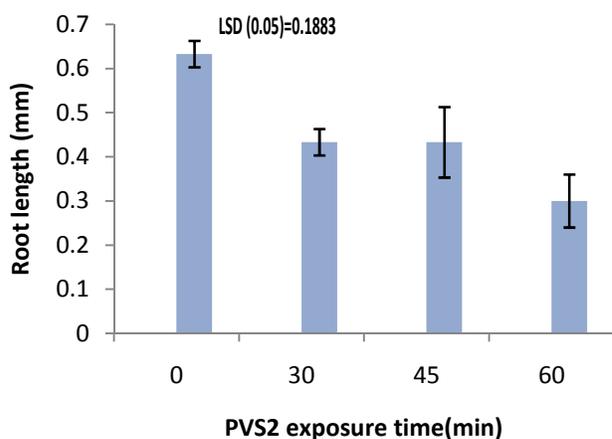


Figure 1: Effect of PVS2 exposure on root length of germinating *Amaranth* seeds. Bars represent standard error of mean.

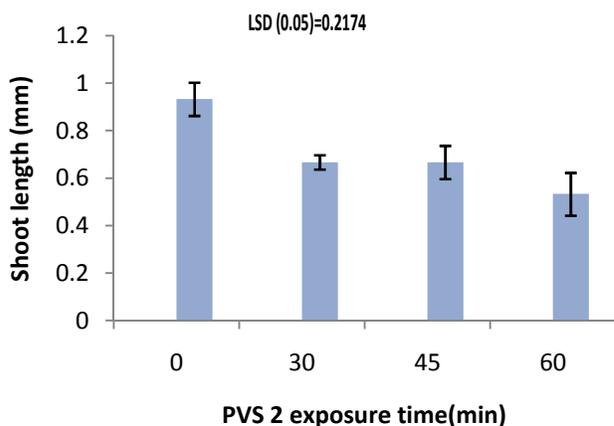


Figure 2: Effect of PVS2 exposure on shoot length of germinating *Amaranth* seeds. Bars represent standard error of mean.

Effect of PVS2 exposure on vigour of *Amaranth* seeds

The PVS2 has significant effect ($P < 0.05$) on the seed vigour (Figure 3). The PVS2 application reduced vigour of the germinating seeds when compared with the control treatment. However, the reduction was not

significantly different among the three exposure periods; 30, 45 and 60 min. Exposure of the seeds to PVS2 for 30 and 45 min inhibited seed vigour by 43.3 % and 37.3 % respectively. This was increased to 62.6 % when the exposure period was extended to 60 min.

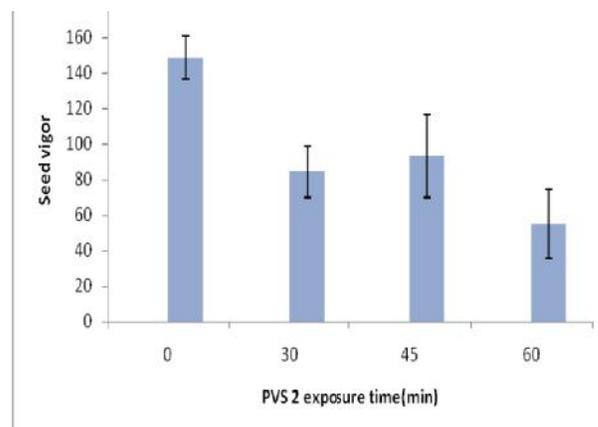


Figure 3: Effect of PVS2 exposure on seed vigour of *Amaranth*. Bars represent standard error of mean.

DISCUSSION

Effect of PVS2 application on percentage germination and moisture of seeds

The results show that germination was not completely inhibited by the PVS2 as two of the exposure times (30 and 45 min) had a good number of seeds germinating (above 75 % germination). Seventy percent (70 %) is the minimum acceptable germination percentage for *Amaranth* seeds locally acquired (FAO, 2010). Therefore exposure periods less than or equal to 45 min will not adversely affect the seeds and they will germinate normally under required germination conditions. The PVS2 dehydrated the moisture of the seeds. The higher the

exposure period the higher the moisture that was removed. Seeds are classified into orthodox and recalcitrant types and *Amaranth* is known to be orthodox and therefore can withstand high dehydration. According to Ellis and Robert (1989), desiccation of orthodox seeds to 2-6 % moisture content does not affect longevity since successful cryopreservation depends on removal of all freezable water in order to avoid ice crystal formation (Nadarajan *et al.*, 2008). In this study, PVS2 exposure period for 45 min removed 50 % of water and this did not adversely affect germination. It is uncertain if this exposure period will remove freezable water and prevent ice crystal formation when the seeds are exposed to liquid nitrogen.

Effect of PVS2 application on vigour of germinating *Amaranth* seeds

The PVS2 applied to the seeds had an effect on root and shoot lengths of the germinating seeds. Root and shoot elongation were inhibited to some extent and the inhibition increased with increasing period of exposure to PVS2, thus reducing the vigour of the seeds. Seed vigor is a component of elongation of the shoot-root lengths and germination which relate with seedling performance (Abdul-Baki and Anderson, 1973). The PVS2 may have some degree of adverse effect on the seed embryo probably

either at the imbibition or emergence stage and longer exposure period may render the seeds less vigorous. Seed lot showing higher seed vigour index is considered to be more vigorous (Abdul-Baki and Anderson, 1973). The relative loss in vigour may be due to increase in solute concentration of cell brought about by over dehydration. Dehydration of tissues prior to cryostorage increases solute concentration of cells which leads to efflux of intracellular water that causes the cells to shrink with change in pH and loss of osmotic responsiveness resulting in membrane injury (Steponkus *et al.*, 1992; Wolf and Bryant, 1999).

CONCLUSION

PVS2 exposure for 45 min or less did not inhibit germination percentage of the seeds of *Amaranth*. However, the root and shoot lengths as well as the vigour of the seedlings were inhibited to a certain extent. PVS2 application for 45 min reduced about half of the moisture content of *Amaranth* seeds and gave 82 % germination and that may be suitable to achieve survival when exposed to liquid nitrogen. However, it will be safer to determine liquid nitrogen exposure period (through further investigations) that will not inhibit both germination and vigour of *Amaranth* seeds.

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