GERMINATION RESPONSE OF AGED SEEDS OF FOUR PEPPER (*Capsicum annuum* L.) GENOTYPES TO HYDROPRIMING

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Abstract
The possibility of hydropriming to bring about reasonable level of re-invigoration and enhancement in seed germination was investigated. Seeds of four pepper genotypes were harvested at 32, 36, 40, 44, 48 and 52 days after anthesis (DAA); the seeds were subjected to eight weeks of accelerated ageing in open plastic plates at 30 ºC and about 85% Relative Humidity inside an incubator. Hydropriming was done by soaking 250 seeds from each of the harvesting stages in 25 ml of distilled water for 12 and 24 hours. After hydropriming, seeds were dried back on paper for 24 hours at room temperature before they were tested for germination alongside the unprimed (control) seeds. Germination was tested by placing four replicates of 50 seeds each on distilled water-moistened filter paper in Petri dishes at 30 ºC for 16 days using the CRD method. Data collected were subjected to Analysis of Variance using the Minitab version 7.0. Means were separated by DMRT. GP increased significantly from 2.17 at 32 DAA to 22.71 at 52 DAA. The values recorded in 52 (22.71%) and 48 (19.29%) DAAs were not significantly different from each other but both were significantly higher than those of the lower ages each of which was also significantly higher than the age below it. The highest Germination Percentage (GP) of 22.06 was recorded in seeds of genotype 4 (V4) which was significantly higher than those recorded in seeds of other genotypes each of which was also significantly greater than the one next to it as follows: 14.60. 7.67 and 5.58% for V2, V1 and V3 respectively. Though hydropiming of seeds brought about significant enhancement in germination compared to the unprimed seeds, the enhancement was marginal. It is therefore concluded that hydrpriming seeds of these genotypes that have already deteriorated is not necessary.

Key words: Hydropriming, Seeds, genotype, Days after anthesis, germination percentage

INTRODUCTION

Priming is a seed technology in which seeds are hydrated for a limited time and re-dried back to the primary moisture level. This technology is known to allow all pre-germinative metabolic processes to take place while preventing radicle protrusion (Farooq *et al.*, 2011). It is described as a simple and cost-effective pre-sowing treatment which improves germination and synchronizes early and vigorous stand establishment in most horticultural crops (Afzal *et al.*, 2002). Priming has been reported to accelerate seed germination and seedling

establishment under both normal and harsh environments (Ashraf and Foolad, 2005). The technology has been adapted in many developed countries to improve seed performance through increased seed vigour subsequent to enhanced percentage of seed germination and rates of seedling emergence (Abdulrahmani *et al*., 2007). The promotive effect has been reported to be due to physiological and biochemical changes that take place during the seed priming treatments which allow seeds to begin the germination sequences before sowing (Hossein *et al.,* 2001).

Priming has been reported to contribute significantly to improvement in seed germination and seedling growth in different plant species (El-Tayeb, 2005; Hayati *et al.,* 2005; Tzortzakis, 2009; Patade *et al.,* 2011). The resultant effect of priming depends on the method used and time of treatment (Hossein *et al.,* 2011). The works of Ashraf and Foolad (2005) and Ghassemi-Golezani *et al*. (2008) reveal that the positive effects of seed priming on seed invigoration depends on priming duration. Ghassemi-Golezani *et al*. (2010) reported the efficacy of hydro-priming for 7 and 14 hours in enhancing seed and seedling vigour, stand establishment and grain yield of pinto bean cultivars in the field. Tilahun *et al.* (2013) reported that planting hydro-primed rice seeds that were soaked for 24 hours and re-dried for the same duration resulted in the highest grain yield of the crop compared to other soaking and drying duration under moisture stress.

Szafirowska *et al.* (2002) reported that priming of aged onion seeds not only improved germination capacity but also led to full restoration of the cell detoxifying mechanisms which were strongly altered during ageing. This according to many authors could be linked with the repairing and building up of nucleic acids, increased synthesis of proteins as well as the repairing of membranes (McDonald, 2000). It was also shown that seed priming causes

metabolic changes in germinating seed, such as cell cycle related events (De Castro *et al*., 2000), endosperm weakening by hydrolase activities (Bradford *et al*., 2000) and mobilization of storage proteins (Job *et al*., 2000). It has also been found to enhance the activities of anti-oxidative enzymes in treated seeds (Wang *et al.*, 2003). Moreover, Chiu *et al.* (2006) reported that both anti-oxidation and lipid-carbohydrate conversion enhancements are involved in priming leading to improved emergence of *Echinacea purpurea* seeds.

Seed deterioration according to Jyoti and Malik (2013) refers to a natural process in seeds involving changes which could be cytological, physiological, biochemical and physical which in turn result in reduced viability and death of the seed. This phenomenon according to Kibinza *et al*. (2006) is associated with various cellular, metabolic and chemical alterations including chromosome aberrations and damage to the DNA, impairment of RNA and protein synthesis, changes in the enzymes and food reserves and loss of membrane integrity.

The aim of this study was to determine the germination and vigour enhancement potency of hydropriming on deteriorated seeds of four genotypes of pepper commonly grown in Minna, Niger State of Nigeria.

MATERIALS AND METHODS

Seeds of four pepper genotype were harvested at 32, 36, 40, 44, 48, and 52 DAA. The seeds were subjected to accelerated ageing for eight weeks (using high storage temperature (35 ^{O}C) and relative humidity of about 85%) in the laboratory of Crop Production Department of Federal University of Technology, Minna. The aged seeds were primed by soaking 250 seeds from each of the harvesting stages in 25 ml of distilled water for 12 and 24 hours. Following priming, the solutions were decanted and the seeds were thinly spread on paper to dried back for 24 hours at room temperature (30 ^{O}C) before they were tested for germination alongside

the unprimed (control) seeds. Germination was tested by placing four replicates of 50 seeds each on distilled water-moistened filter paper in plastic Petri dishes at 30 ºC for 16 days. The number of normal seedlings was expressed as a percentage of the total number of seeds sown to determine germination percentage. Germination rate index (GRI), an estimation of the percentage of seed germination per day and germination index (GI) were also determined using the expressions below:

$$GRI\ (\%\ day^{-1}) = \sum (Ni/i)$$

Where N is the number of seeds germinated on day i.

The higher the GRI value, the higher and faster the germination (Kader, 2005).

GI was calculated using a modification of the formula developed by (Bench, 1991):

$$GI = (16 \times n1) + (14 \times n2) + \ldots\ldots\ldots\ldots + (2 \times n16)$$

Where n1, n2……., n16 are the number of seeds that germinated on the first, second and subsequent days until the 16^{th} day, respectively; 16, 14,…….., and 2 are the weight given to the number of seeds that germinated on the first, second and subsequent days respectively.

GI is assessed by Kader (2005) to be a comprehensive measuring parameter since it combines both germination percentage and speed (spread, duration and high and low events).

RESULTS

The germination percentages (GP) of seeds prior to storage and during storage for two, four and six weeks under accelerated ageing condition is shown in Figure 1. There were general initial increases in the GP of seeds up to 14 days after storage (DAS) irrespective of the genotype and fruit harvesting stages. Following storage for 28 days, a sharp decline was recorded in GP with further downturn to about 22.88% at 42 DAS. Table 1 shows the

germination percentages of 56 days (eight weeks) stored seeds as influenced by hydropriming. The highest GP of 22.06 was recorded for seeds of genotype 4 (V4) which was significantly higher than those recorded in seeds of other genotype each of which was also significantly greater than the one next to it as follows: 14.61. 7.67 and 5.58% for V2, V1 and V3 respectively. GP increased significantly with seed age from 2.17 at 32 DAA to 22.71 at 52 DAA. The values recorded in seeds harvested at 52 (22.71%) and 48 (19.29%) DAAs were not significantly different from each other. The lowest GP of 9.83 was recorded in unprimed seeds which was statistically similar to 11.56% recorded when seeds were hydroprimed for 12 hours. 24-hour hydropriming resulted in the maximum GP (16.04%) which was significantly higher than the values obtained in the other priming treatments (unprimed and 12 hours hydropriming).

Though GP was generally lowest and highest in unprimed and 24-hour hydroprimed seeds respectively, no significant difference was obtained among the priming treatments at 32 to 44 DAA. At 48 and 52 DAA, 24-hour hydropriming resulted in a significantly higher GP than the unprimed seeds but were statistically similar to GP of 12-hour hydroprimed seeds. Hydropriming seeds for 12 hours produced germination values which was statistically similar to the unprimed seeds. In all the priming treatments, GP increased from 32 to 52 DAA such that each age had a GP that was statistically similar to it immediate younger age but significantly higher than those of every other younger age except at 36 DAA where GP was significantly higher than that of 32 DAA (Table 2).

No significant difference was obtained among the GP of the three priming treatments in V1 and V3. However, in V2 and V4, 24-hour hydroprimed seeds germinated significantly higher than the 12-hour hydroprimed lots. Seeds of V4 were significantly higher in GP than the GP values in other genotypes irrespective of the priming treatments while seeds of V2 was also

significantly higher in GP than V1 and V3 both of which were generally statistically similar in GP. The descending order of significance in GP of 24-hour hydroprimed seeds is as follows (V4=29.75, V2= 20.08, V1= 8.08 and V3= 6.25%) (Table 3).

The lowest germination rate index (GRI) 0f 0.35%$^{day-1}$ was recorded in seeds of genotype 3 (V3) which was statistically similar to GRI value of 0.52%$^{day-1}$ recorded in seeds of V1. Seeds of V4 recorded the highest GRI of 1.62%$^{day-1}$ which was significantly higher than the values obtained from seeds of other genotypes. When seeds were extracted from fruit harvested at 32 DAA, the lowest GRI of 0.14%$^{day-1}$ compared to other seed ages was recorded. This value increased significantly to a maximum of 1.60%$^{day-1}$ at 52 DAA. The 1.41%$^{day-1}$ recorded at 48 DAA was statistically similar to the 1.60%$^{day-1}$ obtained at 52 DAA; both values were significantly higher than those of earlier ages.

Umprimed seeds had the lowest GRI (0.60%$^{day-1}$) while seeds hydroprimed for 24 hours resulted in the highest GRI (1.23%$^{day-1}$) which was significantly greater than those of other priming treatments; 12-hour hydroprimed seeds was also significantly higher in GRI (0.78%$^{day-1}$) than that of unprimed lots (0.60%$^{day-1}$) (Table 1). The GRI value obtained from seeds extracted at all the DAAs and hydropriming treatments are generally not significantly different from each other for genotypes 1, 2 and 3. However, when seed harvesting was delayed to 48 and 52 DAA, hydropriming for 24 hours resulted in significantly higher GRI values than the other priming treatments in genotype 4 (Table 4).

Germination index (GI) was lowest (19.74) in seeds of V3 and highest in seeds of V4 (88.58). Each value is significantly higher than each other in the following trend: V4>V2>V1>V3. Seeds extracted from fruit harvested at 32 DAA had the lowest GI of 8.29 which was significantly lower than those recorded in older ages, while the seeds of 52 DAA recorded the highest GI of 88.92 which was significantly higher than those recorded at earlier ages. There

were significant increases in GI from 0.00 in V2 and V3 for 12-hour hydropriming as well as V3 unprimed seeds at 32 DAA to 217 in V4 for 24-hour hydroprimed seeds at 52 DAA. V4 and V2 for 24-hour hydropriming at 48 and 52 DAA respectively were however statistically similar in GI to that of V4 24-hours hydropriming at 52 DAA (Table 5).

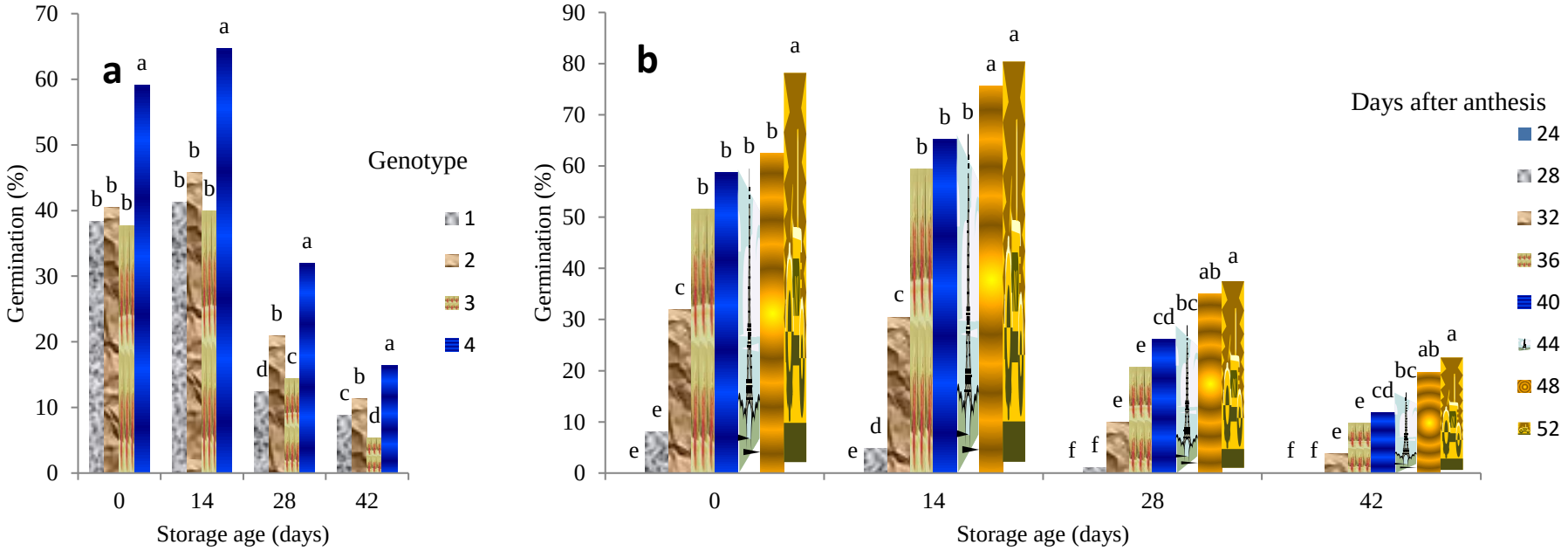

Figure 1: Effect of Genotype (a) and Fruit Age (b) on Seed Germination Percentage (GP) at Different Storage Periods

Means followed by the same letter on the same sets of bars are not significantly different at 5% level of probability according to Tukey Test.

Table 1: Effect of Genotype, Fruit Age and Priming Methods on Germination Percentage, Germination Rate Index and Germination Index of Seeds Subjected to 56 Days Accelerated Ageing.

Treatments	Parameters		
	GP (%)	GRI (% day-1)	GI
Genotype (V)			
1	7.67c	0.52c	29.64c
2	14.61b	1.00b	53.64b
3	5.58d	0.35c	19.74d
4	22.056a	1.62a	88.58a
SE±		0.05	2.41
Fruit age (DAA)			
32	2.17e	0.14d	8.29e
36	6.83d	0.46c	25.60d
40	10.00c	0.66c	37.50d
44	13.88b	0.95b	51.63c
48	19.29a	1.41a	75.46b
52	22.71a	1.60a	88.92a
SE±		0.06	2.95
Priming			
Unprimed	9.83b	0.60c	34.49c
Hydro 12	11.56b	0.78b	44.93b
Hydro 24	16.04a	1.23a	64.98a
SE±		0.04	2.09
Genotype*DAA	NS	*	*
Genotype*Priming	*	*	*
DAA*Priming	*	*	*
Gen*DAA*Priming	NS	*	*

Means followed by the same letter (s) are not significantly different at 5% level of probability by Tukey test.
Hydro 12 = Hydropriming for 12 hours
Hydro 24 = Hydropriming for 24 hours

Table 2: Interaction Effects of Fruit Age and Priming on Seed Germination Percentage

Priming	Days after anthesis					
	32	36	40	44	48	52
Unprimed	2.38j	5.38hi	7.88gh	11.00e-g	15.38c-f	17.00b-d
Hydro 12	1.00j	6.88gh	10.88f-h	12.13d-g	17.13b-e	21.38a-c
Hydro 24	3.13ij	8.25gh	11.25d-g	18.50b-e	25.38ab	29.75a

Means followed by the same letter (s) are not significantly different at 5% level of probability by Tukey test.
Hydro 12 = Hydropriming for 12 hours
Hydro 24 = Hydropriming for 24 hours

Table 3 Interaction Effects of Variety and Priming on Seed Germination Percentage

Priming	Variety			
	1	2	3	4
Unprimed	7.25e-g	10.67de	5.17fg	16.25bc
Hydro 12	7.67e-g	13.08cd	5.33g	20.17b
Hydro 24	8.08d-f	20.08b	6.25g	29.75a

Means followed by the same letter (s) are not significantly different at 5% level of probability by Tukey test.
V1 = Tatashe Dan Kano
V2 = Tatashe Dan Kaduna
V3 = Tatashe Dan Zaria
V4 = Shombo
Hydro 12 = Hydropriming for 12 hours
Hydro 24 = Hydropriming for 24 hours

Table 4: Interaction Effects of Genotype, Fruit Age and Priming on Seed Germination Rate Index

		Days after anthesis					
		32	36	40	44	48	52
Genotype							
V1	Unprimed	0.06mn	0.23l-n	0.30j-n	0.47h-n	0.81g-n	0.87f-n
	Hydro 12	0.07mn	0.36i-n	0.35j-n	0.56g-n	0.72g-n	0.81g-n
	Hydro 24	0.10mn	0.40i-n	0.39i-n	0.68g-n	0.96f-n	1.16f-n
V2	Unprimed	0.12mn	0.25k-n	0.42i-n	0.74g-n	1.06f-n	1.17f-n
	Hydro 12	0.00n	0.53h-n	0.79g-n	0.83f-n	1.19f-n	1.76c-g
	Hydro 24	0.11mn	0.68g-n	1.02f-n	1.59d-i	2.65b-d	3.05ab
V3	Unprimed	0.00n	0.10mn	0.15mn	0.22mn	0.51h-n	0.60g-n
	Hydro 12	0.00n	0.21mn	0.22mn	0.22mn	0.57g-n	0.78f-n
	Hydro 24	0.09mn	0.31j-n	0.30j-n	0.34j-n	0.70g-n	0.93f-n
V4	Unprimed	0.41i-n	0.70g-n	1.05f-n	1.24e-m	1.52d-j	1.46d-l
	Hydro 12	0.22mn	0.86f-n	1.47d-k	1.66c-h	2.05b-f	2.45b-e
	Hydro 24	0.55g-n	0.91f-n	1.50d-j	2.83bc	4.15a	4.19a
SE±				0.21			

Means followed by the same letter (s) are not significantly different at 5% level of probability by Tukey test.
V1 = Tatashe Dan Kano
V2 = Tatashe Dan Kaduna
V3 = Tatashe Dan Zaria
V4 = Shombo
Hydro 12 = Hydropriming for 12 hours
Hydro 24 = Hydropriming for 24 hours

Table 5: Interaction Effects of Genotype, Fruit Age and Priming on Seed Germination Index

		Days after anthesis					
		32	36	40	44	48	52
Genotype							
1	unprimed	3.50jk	12.50h-k	16.50h-k	25.50g-k	46.00f-k	54.50f-k
	Hydro 12	4.00jk	20.50h-k	20.00h-k	30.50g-k	41.00f-k	51.50f-k
	Hydro 24	6.00jk	21.50h-k	24.00g-k	39.00f-k	53.50f-k	63.50e-j
2	unprimed	6.00jk	14.00h-k	22.00h-k	44.00f-k	60.00e-k	67.50e-i
	Hydro 12	0.00k	28.00g-k	42.00f-k	44.50f-k	67.50e-i	94.00c-f
	Hydro 24	6.00jk	39.50f-k	56.50e-k	84.50d-g	135.00cd	154.50bc
3	unprimed	0.00k	3.75jk	8.00i-k	12.00h-k	28.50g-k	39.50f-k
	Hydro 12	0.00k	11.00h-k	13.50h-k	12.50h-k	35.00f-k	47.50f-k
	Hydro 24	4.50jk	17.50h-k	16.00h-k	19.00h-k	34.00f-k	53.00f-k
4	unprimed	24.00g-k	38.50f-k	62.50e-j	69.50e-h	85.00d-g	84.50d-g
	Hydro 12	13.00h-k	49.00f-k	85.00d-g	94.50c-f	117.00c-e	140.00cd
	Hydro 24	32.50g-k	51.50f-k	84.00d-g	144.00b-d	203.00ab	217.00a
SE±				10.2			

Means followed by the same letter (s) are not significantly different at 5% level of probability by Tukey test.
V1 = Tatashe Dan Kano
V2 = Tatashe Dan Kaduna
V3 = Tatashe Dan Zaria
V4 = Shombo

Discussion

The variation in germinability among seeds of different pepper genotypes as recorded in this study is a known phenomenon which has been documented in previous studies. Earlier study conducted by Omotosho (2014) reported that *Capsicum annum* and *Capsicum frutescence* were superior in viability and seedling vigour than *Capsicum chinense* cultivar of pepper. Aloui *et al.* (2014) worked on three cultivars of pepper and reported that Anaheim Chili germinated better than Beldi and Baklouti cultivars. The work of Seyed and Naser (2012) revealed significant differences among ten cultivars of *Solanum lycopersicum* in all parameters including germination percentage.

The increases in GP, germination rate index (GRI) and germination index (GI) with increase in maturity could be attributed to greater inflow of assimilate with progress in seed maturation as reported by Chen *et al*. (2009). Kavak *et al*. (2012) reported that timing of harvest is an important factor since both early and late harvest reduces seed quality.

Enhanced germination percentage, germination rate index and germination index of aged seeds by hydropriming in this study suggests repair of cells and the re-invigoration ability of hydropriming which is in agreement with what has been reported by many workers. Szafirowska *et al.* (2002) reported that priming of aged onion seeds not only improved germination capacity but also led to full restoration of the cell detoxifying mechanisms which were strongly altered during ageing. Invigoration of tomato seeds by priming resulted in higher germination compared to control (Hamdolla, 2012). The effects of priming have also been linked with the repairing and building up of nucleic acids, increased synthesis of proteins as well as the repairing of membranes (McDonald, 2000).

Reasons have been given to explain the effectiveness of priming. Primed seeds have more time to complete the process of repair of damaged DNA because water uptake is slower in priming (Varier *et al*., 2010). Rapid germination in primed seeds can be due to the increasing activity of the degrading enzymes such as α- amylase, synthesis of RNA DNA, ATP and the number of mitochondria. Evidently priming increases free radical scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase in seeds (Afzal *et al*., 2006). Priming improves mean germination time by accelerating imbibition, which facilitate the emergence phase and the rapid multiplication of radicle cells (Kaya *et al*., 2006).

Seed hydroprimed for 24 hours in this study resulted in significantly higher performance when compared to that of 12-hour hydroprimed seed. Soaking duration has been reported to be one of the factors influencing the success of hydropriming. Tilahun *et al.* (2013) reported that planting hydro-primed rice seeds that were soaked for 24 hours and re-dried for the same duration resulted in the highest grain yield of the crop compared to other soaking and drying duration under moisture stress. Ghassemi-Golezani *et al*. (2010) reported the efficacy of hydro-priming for 7 and 14 hours in enhancing seed and seedling vigour, stand establishment and grain yield of pinto bean cultivars in the field.

Conclusion and recommendation

It is concluded in this study that hydropriming significantly enhanced germinability and vigour of aged pepper seeds. This enhancement is however a marginal one which may be crop dependent. Therefore hydrpriming seeds of these genotypes that have already deteriorated is not necessary.

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