

The Effect of Ascorbic Acid Treatment on Viability and Vigor Maize (*Zea mays* L.) Seedling under Drought Stress

HAMIDAH HAMAMA, ENDANG MURNIATI*

Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University,
Darmaga Campus, Bogor 16680, Indonesia

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This study was conducted in the laboratory and the field to examine the effects of ascorbic acid treatment on germination and seedling growth under drought stress. The laboratory works consisted of two experiments and were designed to determine the critical osmotic potential of maize and to determine the optimum ascorbic acid concentration. The field study was designed to examine the effects of soaking seed in ascorbic acid on seedling growth under drought stress. Drought condition was simulated by PEG-6000 and regulation of water treatment. During the first experiment, interactions of both osmotic potential and varieties were significant at all variables. Germination percentage and speed of germination were significantly decreased by increasing of osmotic potential. The second experiment showed that interactions of both factors were significant at all variables except vigor index, the length of shoot, primary, and seminal root. The results showed that the ascorbic acid treatment improved the germination percentage, the speed of germination and the vigor index compared with the control, besides the increase in length of shoot, primary and seminal root and number of seminal root. However, the best result was showed by 55 mM ascorbic acid. The result of field experiment showed that interactions were not always significant and 55 mM ascorbic acid treatment increased the seedling height, the number of leaves and leaf area but it had no effect on the water deficit and the root length.

Key words: ascorbic acid, drought stress, vigor, polyethylene glycol-6000, *Zea mays* L.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important crops in Indonesia. Commonly it is known as a forages crop but in East Indonesia it is known as the main crop at most. Maize is relatively resistant to drought stress, its performance and productivity, nevertheless, can significantly decrease due to drought stress. Drought has different effects on maize depending on the development stage at which it occurs. Drought would induce the production of oxygen reactive species (Arora *et al.* 2002), at the germination stage drought could decrease shoot and root length (Okcu *et al.* 2005), at vegetative period it would decrease shoot growth (Schuppler *et al.* 1998), and cause dehydration on cell and osmotic imbalance (Mahajan & Tuteja 2005). At the silking period it would decrease harvest index until 27.9% and at the grain filling period it would decrease harvest index up to 35.5% (Abo-El-Kheir & Mekki 2007). Priming is one of the solutions to reduce drought effect besides using drought tolerance varieties. Seed priming is widely used for enhancing seed performance by improving the rate and uniformity of germination and decreasing seed sensibility to external factors (Corbineau & Côme 2006). The benefits of seed priming has been reported, including improve stand establishment at semi arid condition (Clark *et al.* 2001) and at drought stress (Kaur *et al.* 2002), enhancing seed

with low vigor (Afzal *et al.* 2004; Bittencourt *et al.* 2005), improving dormancy breakdown (Farooq *et al.* 2005), or increasing productivity (Hussein *et al.* 2007).

Ascorbic acid is one of the most important antioxidant at cellular processes including cell division and expansion, and at metabolism activity when germination started (Arrigoni *et al.* 1992), cell detoxification, protecting cell from reactive oxygen species and preventing death cell (Conklin & Barth 2004). Pre-sowing treatment with ascorbic acid is widely used. It improves performance and stand establishment at different external factors such as high salinity (Shaddad *et al.* 1990; Afzal *et al.* 2005), nematode infection (El-Zawahry & Hamada 1994). Ascorbic acid treatment has been reported on improving performance and stand establishment on direct seeded fine rice (Farooq *et al.* 2006), or transplanted rice (Farooq *et al.* 2007).

The problem is environment stress like drought, which effect germination and seedling Growth especially on low vigor seeds. Ascorbic acid treatment will solve such problem.

MATERIALS AND METHODS

This study was carried out at Seed Science and Technology Laboratory, Department of Agronomy and Horticulture, Soil Physic Laboratory, Department of Soil Science and Land Resource, Faculty of Agriculture, Bogor Agricultural University from March until October 2008. Maize seed var. Arjuna and Bisma for this study was

*Corresponding author. Phone/Fax: +62-251-8629353,
E-mail: endangm.sutirto@yahoo.com

obtained from Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development. We conducted the study in the laboratory as well as in the field.

Laboratory Research. This research consisted of two experiments. The first experiment was conducted to determine the critical moisture content of maize seed and the second one was conducted to determine ascorbic acid concentrations would be used at field research.

The Effect of Drought Stress on Maize Seed Viability. Completely randomized block design with two factors and three replications were used in this experiment. The first factor was osmotic potential levels (0, -0.2, -0.4, -0.6, -0.8, and -1.0 MPa), the second one was varieties (Arjuna & Bisma). Five levels of drought stress condition were simulated by polyethylene glycol-6000 treatment which was arranged as described by Michael and Kaufmann (1973). The higher concentration of PEG is used, the more extreme drought is obtained. Seeds of each variety were germinated at straw paper substrates on germinator IPB 72-1. Germination percentage was recorded at 5 and 7 days after sowing (DAS), speed of germination was recorded based on %/etmal which etmal is 24 hours as described by Sadjad *et al.* (1999). The critical of substrate moisture content for the next experiment was determined from the result of first experiment; and the result showed that critical media moisture content was -0.6 MPa showing fewer than 50% germination percentage. The critical of substrate moisture content was then converted into pF to determine the field-capacity moisture content. And the result showed that -0.6 MPa was equivalent to pF 3.78.

The Effect of Ascorbic Acid Treatment on Maize Seed Vigor under Drought Stress. Completely randomized block design with two factors and three replications were used in this experiment. The first factor was varieties (Arjuna and Bisma) and the second one was ascorbic acid levels (0, 55, 110, 165, 220, and 275 mM). Seeds were soaked at ascorbic acid solution for 24 hours and the seeds were then washed three times with distilled water and re-dried to their original moisture content on a tissue paper for 48 hours at room temperature. The ratio of seed weight to volume of ascorbic acid solution was 1:5 (w/v) (Basra *et al.* 2006). The seeds of each variety were germinated at a straw paper substrate on the germinator IPB 72-1. Germination percentage was recorded at 5 and 7 DAS, speed of germination was recorded as described by Sadjad *et al.* (1999), vigor index was recorded at 5 DAS as described by Copeland and McDonald (2001), the length of shoot, seminal and primary root and number of seminal root were recorded at 7 DAS.

Field Research: The Effect of Ascorbic Acid Treatment on Maize Seedling Vigor under Drought Stress. This Experiment was arranged in two factors completely randomized block designed with three replications and 14 plants per replicate. The first factor was varieties (Arjuna and Bisma) and the second was ascorbic acid level (0 and 55 mM). Media used in this experiment was latosol soil, sand and compost. The ratio of each component was 3:2:1(v/v). The moisture content of air-dried media of pF 2.54 and pF 3.78 analyzed by IPB

soil physic laboratory was 27.73, 41.57, and 30.50%. These moisture contents were evaluated to determine the amount of water irrigated to each polybag to keep the media moisture content at pF 3.78. At 1-3 weeks after planting (WAP) the absolute dry weight of the media was 1.5 kg and at 4-8 WAP the absolute dry weight of the media was 6.5 kg. At 1-3 WAP each polybag was irrigated until reaching 1.92 kg and at 4-8 WAP it was irrigated until reaching 8.5 kg. A balance was used when irrigating the bags to the correct weight. Seedling height and number of leaves were recorded every week, leaf area and water deficit were recorded every two weeks. Leaf area was recorded as described by Sitompul and Guritno (1995) and water deficit was recorded as described by Barrs (1968). Root length was recorded at 8 WAP.

Data were subjected to analysis of variance and differences between means separated by Duncan's test ($P=0.05$).

RESULTS

Laboratory Research : The Effect of Drought Stress on Maize Seed Viability and The Effect of Ascorbic Acid Treatment on Maize Seed Vigor under Drought Stress.

Table 1 showed that the germination percentage (GP) and the speed of germination (SG) of both varieties were not significantly decreased at -0.2 MPa. It showed also that Bisma had a better vigor than Arjuna. Germination percentage and SG of Arjuna and Bisma were significantly decreased at -0.4 MPa but Bisma has a higher GP (87.7%). GP and SG were significantly decreased by increasing osmotic potential of PEG. At -0.6 MPa osmotic potential, GP and SG of both varieties were lower than the lowest recommended standard of viability (i.e. 60%). It seemed that the critical osmotic potential of both varieties were -0.6 MPa.

A significant of two ways interaction (variety and AA levels) was found ($P < 0.05$) for GP, SG, primary root length at 5 DAS (PRL-5DAS) and number of seminal root (NSR) at the second experiment of the laboratory research. Table 2 showed that the best result was obtained from the seeds treated with 55 mM ascorbic acid at all variables of both varieties. The increasing of ascorbic acid level more than 55 mM did not affect all investigated characters. The characters are germination percentage (GP), speed of germination (SG), primary root length (PRL), number of seminal root (NSR), seminal root length (SRL), shoot length (SL), and vigor index (VI) (Table 2 & 3).

Table 1. Germination percentage (GP) and speed of germination (SG) Arjuna and Bisma under different drought levels

Variety	Osmotic potential (MPa)					
	0	-0.2	-0.4	-0.6	-0.8	-1
	Germination percentage (%)					
Arjuna	100a	98.7a	54.7d	56.7c	8.7e	0f
Bisma	100a	96.0a	87.7b	50.7d	0f	0f
	Speed of germination (%/etmal)					
Arjuna	31.53a	26.33b	8.69d	7.05d	1.33e	0e
Bisma	32.25a	26.90b	15.70c	8.25d	0e	0e

Means followed by the same letter are not different by the Duncan's test ($P < 0.05$).

Field Research: The Effect of Ascorbic Acid Treatment on Maize Seedling under Drought Stress.

Table 4 showed that the seedling height (SH) and the number of leaves (NL) of Arjuna and Bisma were not different to control (0 mM AA). Arjuna responded AA treatment since the first week of drought stress but Bisma responded since the fourth week. At the fourth week Bisma responded AA treatment as significant as Arjuna. At the fourth week, AA treatment increased NL of both varieties significantly. Ascorbic acid treatment and varieties or both

interactions did not influence root length. Water deficit were not different at both varieties in control. The leaves area were significantly influenced by AA treatment. The leaves area was 386.54 cm² at control and it was 549.38 cm² at 55 mM AA treatment.

DISCUSSION

Laboratory Research. Germination percentage (GP) and the speed of germination (SG) of Arjuna decreased significantly at -0.4 MPa and Bisma was at -0.6 MPa. At -0.6 MPa osmotic potential level, GP and SG of both varieties were lower than the lowest recommended standard of viability. It showed that critical osmotic potential of both varieties were -0.6 MPa. These results are in agreement with Rahimi *et al.* (2006) that drought stress created by PEG 6000 at -0.6 and -0.8 MPa have decreased GP until 24.5 and 12.2% on *Plantago ovata* seed. Okçu *et al.* (2005) has found that -0.6 MPa osmotic potential decreased *Pisum sativum* seed vigor and its reduction was more significant on seed with low vigor. The obstruction of seed germination caused by drought stress would decrease SG, because water availability influences seed metabolism. Bewley and Black (1983) found that drought stress would decrease enzyme activity and seed metabolism. Its decrease would inhibit cell division and cell expansion. As a result, shoot and root growth on germination would be inhibited.

Table 2. Interaction of ascorbic acid level and variety on germination percentage, speed of germination (SG), primary root length (PRL) at 5 day after sowing (DAS) and number of seminal root (NSR)

Variety	Ascorbic acid (mM)					
	0	55	110	165	220	275
Germination percentage (%)						
Arjuna	50.7c-d	68b	64bc	60b-d	45.3d-f	52c-e
Bisma	41.3e-f	88a	36ef	69.3b	50.7c-e	30.7f
Speed of germination (%/etmal)						
Arjuna	8.75d-f	12.21bc	11.07b-d	10.13c-e	8.72d-f	8.15e-g
Bisma	6.81f-h	16.38a	7.54e-h	12.94b	5.56gh	5.34h
Primary root length at 5 days after sowing (cm)						
Arjuna	8.21d	10.03ab	8.76cd	8.33d	6.69e	8.64cd
Bisma	10.80a	9.64bc	9.02b-d	8.06d	8.98b-d	9.21b-d
Number of seminal root						
Arjuna	6.4d	9.75a	8.79b	7.90c	8.27bc	7.61c
Bisma	7.6c	7.79c	7.48c	7.86c	6.7d	8.01bc

Means followed by the same letter are not different by the Duncan's test ($P < 0.05$).

Table 3. Ascorbic acid's influences on vigor index, primary root at 7 days after sowing, seminal root (SRL) and shoot length (SL)

Variety	Ascorbic acid (mM)						y
	0	55	110	160	220	275	
Vigor index (%)							
Arjuna	16.00	40.00	10.67	16.00	17.33	20.00	20.00
Bisma	12.00	45.33	9.33	21.33	13.33	16.00	19.56
X	14.00bc	42.67a	10.00c	18.67b	15.33bc	18.00bc	
Primary root length at 7 after counting							
Arjuna	12.24	12.09	10.52	10.59	9.19	10.78	10.91
Bisma	13.08	13.06	11.27	11.77	10.98	11.68	11.98
X	12.66a	12.58a	10.90bc	11.18b	10.09c	11.23b	
Seminal root length (cm)							
Arjuna	7.79	9.81	9.16	8.97	7.93	7.77	8.56
Bisma	8.54	12.18	11.01	10.99	9.04	9.48	10.21
X	8.16c	10.99a	10.06b	9.98b	8.48c	8.62c	
Shoot length (cm)							
Arjuna	2.27	3.5	2.69	3.32	2.79	3.54	3.06
Bisma	2.31	4.55	2.92	3.76	3.25	3.54	3.35
X	2.29d	4.02a	2.81cd	3.54ab	3.02bc	3.54ab	

Means follow with the same letter at each row are not different by the Duncan's test. X = mean influenced by ascorbic acid; y = mean influenced by variety.

Table 4. Influences of ascorbic acid treatment on seedling height and number of leaves at 1, 2, 3 and 4 weeks after drought stress condition

Variety	Ascorbic acid (mM)							
	1 WAS		2 WAS		3 WAS		4 WAS	
	0	55	0	55	0	55	0	55
Seedling height (cm)								
Arjuna	92.97b	105.18a	113.59b	135.11a	135.23b	150.96a	150.69c	172.31a
Bisma	98.08b	90.81b	119.92b	116.58b	138.47b	135.34a	150.29c	160.52b
Number of leaves								
Arjuna	6.24b	7.81a	7.67b	9.44a	8.44c	10.48a	9.33c	11.81a
Bisma	7.17ab	7.03ab	8.41ab	8.22ab	8.59c	9.37b	9.19c	10.65b

Means followed by the same letter are not different by the Duncan's test ($P < 0.05$); WAS = weeks after stress.

GP of both varieties at control (0 mM AA) were under 60%. The best result of GP and SG was showed by 55 mM AA treatment. This results agree with Basra *et al.* (2006) that 10 ppm AA treatment before sowing in seed rice cv. KS-282 and Super Basmati had higher GP and better performance than control or 20 ppm AA treatment. GP and SG were significantly reduced by increasing of AA treatment but it was not different with control. It suggested that higher than 55 mM of AA treatment could inhibit maize seed germination under drought stress. Çanakci and Munzuroğlu (2007) found that soaking *Cucumis sativus* seed in salicylic acetyl acid before planting in high concentration (0.5×10^{-2} M) could decrease germination but lower concentration (10^{-3} , 10^{-4} , and 10^{-5} M) could not.

The primary root length at 5 DAS (PRL-5DAS) and number of seminal root (NSR) of Bisma at control was higher than those of Arjuna. Ascorbic acid treatment could improve PRL-5DAS and NSR of Arjuna and the best result was showed by 55 mM. Generally, at 110-275 mM AA treatment did not influence PRL-5DAS and NSR of both varieties. There was no AA treatment that could improve PRL-5DAS and NSR at Bisma. The results have showed that AA treatment in high concentration did not improve PRL-5DAS and NSR. Primary root length at 7 DAS and seminal root length (SRL) of both varieties were significantly different. Bisma had higher PRL-7DAS and NSR than Arjuna. It suggested that Bisma has better vigor at drought stress than Arjuna so Bisma has more root scope growth than Arjuna. This result is in agreement with Ünyayar *et al.* (2005) that drought tolerance species has higher root length than drought sensitive species at drought stress. Okçu *et al.* (2005) has reported that at -0.6 MPa, drought sensitive of seed *Pisum sativum* L. had no root but drought tolerance had. Ascorbic acid treatment did not improve PRL-7DAS. It suggested that AA treatment did not influence PRL but SRL because SRL would have more responsibility at seedling growth than PRL. Ascorbic acid treatment improved vigor index, SRL and shoot length (SL) and the best result was showed by 55 mM AA treatment. These results agree with Shaddad *et al.* (1989) that soaking seed at AA and pyridoxine 50 ppm for 4 hours before planting improved root length of *Lupinus termis* and *Vicia faba* seeds under saline conditions. Dolatabadian and Modaresnavy (2008) reported that AA treatment improved root length of *Brasica napus* L. and *Helianthus annus* L. Afzal *et al.* (2005) reported that soaking seed with 50 ppm AA and salicylic acid for 12 hours before planting improved SL and reduced the adverse effects of nematode infections. Beltagi (2008) showed that 4 mM AA treatment improved SL of *Cicer arietinum* L. seed germinated at 40 mM NaCl.

Field Research. Ascorbic acid treatment and varieties or both interactions did not influence root length but AA treatment improved NL. At germination stage (Laoratory Research) AA treatment significantly influenced root growth but it did not work at field performances. The same result was observed by Beltagi (2008) that soaking seed at 4 mM AA increased NL of *Cicer arietinum* L. germinated at 20 and 40 mM NaCl but it did not increase root length.

In this experiment AA treatment did not influence water deficit because AA treatment could not substitute the loss of water availability at drought stress. Arora *et al.* (2002) reported that at stress condition AA would influence plant metabolism by protecting cell from reactive oxygen species and free radicals produced more during the stress. Reactive oxygen species and free radicals could obstruct cell division and cell expansion and prevent cell death. Media moisture content at -0.6 MPa was 30.5 or 10% less than optimum moisture content. It may explain why water deficit was not different at both treatments. Bai *et al.* (2006) found that water deficit at moderate level of drought stress (55% of Field Capacity) was not different with control, water deficit was significantly different with control at a high level of drought stress (35% FC). Palupi and Dedywiryanto (2008) showed that water deficit *Elais guineensis* L. at low (75% FC) and moderate level of drought stress (50% FC) were not different with control. Water deficit was significantly different at high level of drought stress (25% FC). The increase of leaves area showed that AA treatment significantly improve seedling performance under drought stress because generally drought stress would reduce leaves area. Kim *et al.* (2000) found that some genotypes of *Glycine max* under drought stress have less leaves area than at optimum condition. Hussein *et al.* (2007) showed that pre-sowing treatment with salicylic acid 200 ppm increased leaves area maize seedling under saline stress stimulated with 4000 ppm NaCl.

In conclusion, critical osmotic potential of Arjuna and Bisma was -0.6 MPa. At germination stage ascorbic acid treatment improved seed vigor by increasing GP, SG, VI, PRL-5DAS, PRL-7DAS, SRL, NSR, and SL. The best result was showed by 55 mM AA treatment. At seedling stage 55 mM ascorbic acid treatment improved seedling vigor by increasing SH, NL, and LA.

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