OPEN ACCESS Freely available online

Journal of Horticulture

Research Article

The Effect of Seed Treatments on the Germination of Fresh and Stored Seeds of Okra (*Abelmoschus esculentus*) and Water Spinach (*Ipomoea aquatica*)

Andreas W. Ebert*, Tien-Hor Wu

World Vegetable Center, 60 Yi-Min Liao, Shanhua, Tainan 74151, Taiwan

ABSTRACT

Okra and water spinach are traditional vegetables that are vital for healthy diets. Major variations in germination response have been observed among genebank entries. This study was undertaken to elucidate the effect of various seed treatments on the germination of fresh and stored seed of okra and water spinach. Freshly harvested fruits of two okra and two water spinach accessions with contrasting geographical origin and breeding status were dried in a screenhouse prior to manual seed extraction and cleaning. Cleaned seeds were dried to 6% seed moisture content in a dehumidified drying room. Various seed treatments were conducted after a 6-month storage period at 5°C and -15°C. Our studies confirmed previous results that both okra and water spinach are difficult to germinate and to obtain good field establishment due to physical, seed coat-imposed dormancy. Moreover, significant genotypic variation among genebank entries/cultivars was found for both crops, making germination response difficult to predict. Improved varieties showed much better germination response than landraces and did not require seed treatments for satisfactory germination. Hydropriming, i.e. soaking of seed in water for 24 hours, did not have an impact on seed germination. Partial removal of the seed coat, followed by 24 hours soaking in water consistently resulted in high germination percentages (>80%) of genotypes of both crops that had not undergone dedicated breeding (landraces), under laboratory and screenhouse conditions and during two subsequent growing seasons. When distributing seed samples of semi-domesticated accessions or landraces, genebanks should advise seed recipients that scarification methods may be necessary to obtain satisfactory seed germination and field establishment.

Keywords: Okra; *Abelmoschus esculentus*; Water spinach; *Ipomoea aquatica*; Seed germination; Seed treatments; Stand establishment; Traditional vegetables

INTRODUCTION

Traditional vegetables are often considered as key assets for supporting more nutrition-sensitive agriculture and building more resilient production systems under climate change as they are often better adapted to poor quality soils, have higher resistance to pests and diseases, and higher nutritional values as compared to global vegetables [1,2]. However, despite the wealth of traditional knowledge existing about traditional vegetables, many remain underutilized due to lack of appropriate seed production, drying, storage, and processing technologies, as well as availability and access to quality seed.

The World Vegetable Center (WorldVeg) maintains the world's largest international public collection of vegetable genetic resources at its headquarters in Taiwan. It has accumulated more than 67,000 accessions and sub-accessions comprising 171 genera and 438 species from 156 countries of origin [3]. The WorldVeg genebank includes a global collection of more than 10,000 traditional vegetables from South Asia, Southeast Asia, and Africa [4]. Among the group of traditional vegetables, okra (*Abelmoschus esculentus* (L.) Moench) and water spinach (*Ipomoea aquatica Forsk*) are quite popular in Asia and Africa and genetically diverse seed samples of these crops are often requested from the WorldVeg genebank for the purpose of screening for pest and disease resistance, breeding as well as subsistence or commercial production. However, the feedback from many seed recipients of okra and water spinach samples revealed that seed germination and stand establishment is often poor and highly variable between different accessions indicating a clear genotypic effect. Therefore, we reviewed the existing literature on how germination hurdles of okra and water spinach can be overcome and designed a field experiment to test the effect of different seed treatments on contrasting genotypes.

Correspondence to: Andreas W. Ebert, World Vegetable Center, 60 Yi-Min Liao, Shanhua, Tainan 74151, Taiwan, Tel: +49(0) 7171 8743794, E-mail: ebert.andreas6@gmail.com; tien-hor.wu@worldveg.org

Received: March 10, 2019; **Accepted:** March 25, 2019; **Published:** April 02, 2019

Citation: Andreas W. Ebert, Tien-Hor Wu (2019) The Effect of Seed Treatments on the Germination of Fresh and Stored Seeds of Okra (*Abelmoschus esculentus*) and Water Spinach (*Ipomoea aquatica*). J Hortic 6: 1. doi: 10.4172/2376-0354.1000254

One of the factors responsible for low okra yield is poor stand establishment, resulting from poor and non-synchronous germination [5] with commercial cultivars reaching only up to 66% initial germination in India [6]. Factors like genotype, position of pods on plant, form of pods (angular versus round/cylindrical) and time of harvesting of pods, seed moisture content (SMC) and micronutrient applications have been reported as affecting okra seed germination [5,7,8]. According to Doijode [9], longevity of okra seeds is improved by seed drying and storage at sub-zero temperatures. The Millennium Seed Bank (MSB) of the Royal Botanical Gardens Kew categorizes the seeds of this species as difficult, as they may have physical dormancy, which could be overcome by scarification to facilitate water uptake and enhance germination [10].

Germination of water spinach seeds is reportedly low (<60%) due to hardseededness, which is often enhanced by long storage periods [11]. Given the low SMC and extended storage periods of water spinach seeds in genebanks, induced hardseededness is more pronounced with seeds kept in genebank storage than in commercially traded seeds [12].

Hardseededness is known to occur in species of the Leguminosae family but can also occur in species of other families such as the Malvaceae to which okra belongs [12]. When seeds are dried, they tend to become first reversibly hardseeded (at around 10-12% moisture content) and this can be overcome by exposing the seeds to high relative humidity for a long time. Further drying of the seeds to about 5-7% which is necessary for safe long-term storage of seeds in genebanks leads to irreversible hardseededness [12]. Hard seeds are characterized by impermeable seed coats, therefore cannot adequately imbibe water and therefore fail to germinate.

Different methods have been tested to overcome hardseededness. These include mechanical, chemical, and physical scarification treatments to make the seed coat permeable for water uptake [12,13]. Mechanical scarification can be done manually by rubbing the testa on abrasive paper (sandpaper), puncturing the testa with a sharp needle, cutting a part of the testa off with a sharp blade. Mechanical scarification can also be achieved by blowing the seeds with a blast of compressed air into a metal drum lined with abrasive paper so that the seeds impinge upon the abrasive surface before exiting the cylinder [13]. This procedure can be repeated several times to obtain enough abrasion of the testa.

Chemical scarification treatments consist of soaking and agitating seeds in a range of concentrated acid solutions (sulphuric acid or other acids, vinegar, etc.) for different durations (from a brief dip in acid to several hours), depending on the species. Treatment with concentrated acid is a severe treatment and may damage at least some of the seed if highly concentrated acids or long exposure times are used. Therefore, we opted for the use of vinegar in our study. Physical scarification treatments consist in exposing the seeds to extreme temperatures—either in air or in water—and rapid temperature changes. Temperatures in the range of 50 up to 80 or 90°C are often used with exposure times varying from 2-20 minutes [12,13]. However, these heat treatments work as ageing treatments and should only be used if seeds are immediately sown. Ultra-low temperatures may also be used for physical scarification by plunging dry seed in liquid nitrogen after pre-cooling them overnight [13]. Several short duration dips of about 30 seconds each in liquid nitrogen with one minute in between dips seems to be more effective than a single longer immersion for about five minutes [12].

Other methods which may lower the effect of seed covering structures in reducing the rate of imbibition are pre-soaking and pre-washing seed [12]. Seed priming (controlled hydration followed by seed drying) is a technique to improve the germination behavior of seed, inducing faster and more uniform germination over broader temperature ranges and breaking seed dormancy inherent in certain species. This field study was undertaken to elucidate the effect of various seed treatments on the germination of fresh and/or stored seeds of contrasting genotypes of okra and water spinach.

MATERIALS AND METHODS

Plant materials and growing seasons

Two accessions of each crop with contrasting geographical origin, breeding status (landrace versus improved cultivar), and 1000-seed weight were chosen from the WorldVeg genebank to verify potential genotypic differences (Table 1).

The trials were conducted during two subsequent growing seasons (2012-13 and 2013-14) at the WorldVeg experimental fields in Shanhua (23°06' 77.0" N 120° 17' 83.6" E), southern Taiwan. Production of fresh seed from samples drawn from seed lots stored under long-term conditions (-15°C) at the WorldVeg genebank took place prior to the start of the experiments. The two okra accessions were sown in March 2013 and March 2014 and harvested at weekly intervals according to the ripening stage of the pods in June 2013 and June/July 2014, respectively. Harvesting of pods of accession VI046536 started at 42 days after anthesis (DAA) and lasted up to 65 DAA, while harvest of VI050958 had a longer duration of 40-87 DAA.

The water spinach accessions were sown in September 2012 and fruit harvested in March 2013. Seeds harvested during the first growing season were sown again in October 2013 to assess field establishment and to produce fresh seeds for the 2014 trials. Unfortunately, the relatively late sowing in 2013 prevented flowering during the same year and plants had to be left in the field for one full year until harvesting could be accomplished in October/November 2014.

Table 1: Details of okra and water spinach accessions used in the trials.

Crop	Accession #	1000-seed weight (g)	Country of origin	Status of sample	Year of acquisition
Okra	VI046536	72.0	Thailand	Landrace	1998
	VI050958	64.2	Zambia	Unknown*	2002
Water spinach	VI050476	44.1	Thailand	Unknown	2002
	VI054533	51.7	Taiwan	Breeding line	2003

*Status of sample unknown during acquisition but field performance indicates an improved cultivar

Seed processing, seed moisture content determination, germination tests, packing and storage

There is sequential ripening of okra pods on the plant and the maturity of the seeds is associated with the pods becoming grey in color. Water spinach fruit turn brown in color when mature. Fully mature okra pods and water spinach fruit were harvested and dried for two weeks in a screenhouse, followed by manual seed extraction and cleaning. Air-dried water spinach fruit were mechanically crushed prior to manual seed extraction. Cleaned seed of okra and water spinach were dried for 3 and 8 days, respectively, in a dehumidified drying room of the Genetic Resources and Seed Unit of WorldVeg at 18°C and 10% RH to 6% SMC. A germination test of fresh okra and water spinach seeds was conducted before drying and storage during the second year of the trials.

A quick, non-destructive, reliable and simple method of testing the moisture status of seeds is seed equilibrium relative humidity (eRH), combining the features of oven tests [14] and moisture meters [15]. Seed eRH was determined at room temperature (approximately 28°C) using enough intact seeds to fill a 3.2 ml sample holder, which was placed in the measuring chamber of an AW-D10 water activity station used in conjunction with a HygroLab 3 display unit (Rotronic South East Asia Pte. Ltd., Singapore). Measurements [water activity (= eRH/100) and temperature] were recorded and converted into SMC with the help of an online tool available in the Seed Information Database [16].

Once the target SMC of 6% was reached, sub-samples (four replicates of 25 seeds each) of both crops were used to determine seed germination using the between paper method (BP) as described by Ellis and co-workers [12]. Okra seeds were incubated for 21 days at alternating temperatures of 20/30°C (16 h/8 h), with light provided for 8 hours during the high-temperature cycle. The provision of a photoperiod also enhanced germination of *Hibiscus trionum* seeds [17]. Water spinach seeds were kept for 10 days at a constant temperature of 30°C [18], in a growth chamber (model ST3-2, Saint Tien Co. Ltd.). The remaining seed were quickly packed in small size aluminum foil bags (25 seeds per bag) and air was manually pressed out from the bags before heat-sealing.

Seed storage and seed treatments

Okra and water spinach seed samples packed in aluminium foil bags as described above, were stored in cold rooms operated at 5°C (medium-term storage conditions) and -15°C (long-term storage conditions) for a 6-month period during two subsequent growing cycles.

At the end of each storage period, seed treatments of okra and water spinach were conducted. At the end of the second growing cycle, seed treatments were performed prior to seed storage as well. The following seed treatments were applied to two batches each in four replicates of 25 seed each: T1 - control; T2 - 24 hours soaking in water at room temperature (28 ± 2°C); T3 - partial removal of seed coat with the use of a scalpel, followed by 24 hours soaking in water; T4 - soaking in rice vinegar (≥ 4.5% acidity) for 2 hrs; T5 - soaking in KNO_3 solution (0.3%) for 1 hour; T6 - 24 hours soaking in water, followed by quick surface drying and then drying in a dehumidified chamber for 72 hours. These treatments were chosen for ease of application at family farm level. The germination of treated seed was determined in a growth chamber in the laboratory as described above. A second batch of treated okra and water spinach seed was sown in seedling trays with peat moss and kept in a screenhouse during germination for subsequent field transplanting. Stand establishment (percentage of plant survival in the field) was assessed at three weeks after field transplanting.

During the second growing season, seed treatments of okra were conducted prior to storage, in August 2014, and after a 6-month storage period, in January 2015. Seed priming of water spinach was carried out in January 2015, prior to storage, and after a 6-month storage period, in July 2015.

Data was statistically examined by analysis of variance (ANOVA) using Statistical Analysis Software (SAS) 9.4. Tukey's honest significant difference (HSD) test was used to determine significant differences among treatment means and factors at the $p<0.05$ level.

RESULTS

Okra

Fresh, dried okra seeds of accession VI046536 from Thailand had an initial germination of 26%, prior to storage, compared to 90% of accession VI050958 from Zambia, in 2013 (data not shown). In 2014, germination of fresh, dried and untreated (T1) seeds was much higher, VI046536 reaching 80% germination and VI050958 from Zambia 98% (data not shown). Untreated seeds (T1) of both accessions showed a similar, quite contrasting germination pattern after a 6-month storage period at either 5°C or minus 15°C, measured under laboratory and screenhouse conditions (Tables 2 and 3). Seed germination of the control (T1) of VI050958 was lower under screenhouse conditions compared with laboratory conditions, while this trend could not be confirmed for VI046536 from Thailand.

Partial removal of the seed coat, followed by 24 hours soaking in distilled water (T3) significantly enhanced germination of accession VI046536 to 80-90% in 2013, a level comparable to VI00958, especially after storage at -15°C (Tables 2 and 3). Other seed treatments were not beneficial. This was true for both storage environments and both evaluation environments (laboratory and screenhouse). In 2014, germination of seed exposed to T3 did not statistically differ from the control due to a much higher germination of the latter compared to the previous year. Given the high germination of accession VI050958, prior to storage and after a 6-month storage period (T1), additional seed treatments had no significant beneficial effects. In general, germination was slightly lower under screenhouse conditions compared to laboratory conditions. Apart from achieving satisfactory germination of VI046536 with treatment 3 (T3), this treatment also resulted in the highest plant survival in the field in 2013, for both accessions (Table 3).

Storage conditions did not have a notable impact on germination of both okra accessions under laboratory and screenhouse conditions during both years (Tables 2 and 3).

Water spinach

Fresh, dried water spinach seed of accession VI050476 from Thailand had an initial germination of 4 and 21%, prior to storage, compared to 77 and 73% of accession VI054533 from Taiwan in 2013 and 2014, respectively (data not shown). This contrasting germination behavior of control seed (T1) between both accessions was maintained after a 6-month storage period during two subsequent years (Table 4). Partial removal of the seed coat,

Table 2: Germination (percentage data arcsine transformed) of seed of two okra accessions dried to 6% SMC in drying room, prior to storage and after a 6-month storage period at 5°C and minus 15°C as affected by seed treatments, for two growing seasons. Germination was assessed under laboratory conditions.

Accession Country	Seed treatments	Germination after 6-month storage at 5°C 2013	Germination after 6-month storage at -15°C 2013	Germination prior to storage 2014	Germination after 6-month storage at 5°C 2014	Germination after 6-month storage at -15°C 2014
VI046536 Thailand	FS[a]			46.70		
	T1[b]	24.16 b	26.41 b	64.62 ab	69.03 a	71.65 a
	T2	23.97 b	28.60 b	65.00 ab	70.82 a	77.03 a
	T3	71.78 a	78.83 a	49.11 b	73.84 a	74.79 a
	T4	15.77 b	27.25 b	75.69 a	69.24 a	71.78 a
	T5	24.24 b	26.33 b	67.50 ab	77.85 a	72.23 a
	T6	14.30 b	13.34 c	60.77 ab	68.15 a	69.03 a
VI050958 Zambia	FS[a]			77.30		
	T1[b]	86.86 a	81.62 ab	84.06 a	86.86 a	84.06 a
	T2	81.88 a	74.09 b	81.88 a	77.27 a	85.64 a
	T3	80.66 a	76.01 ab	69.86 ab	82.84 a	84.06 a
	T4	80.66 a	86.86 a	64.62 b	72.61 a	77.24 a
	T5	77.85 a	80.04 ab	84.06 a	86.86 a	89.67 a
	T6	80.04 a	72.87 b	82.84 a	82.84 a	81.26 a

[a]FS = Germination of untreated fresh seed before drying.
[b]Treatment means followed by different letters in a vertical column are significantly different (P<0.05) from each other, based on Tukey's HSD test.

Table 3: Germination (percentage data arcsine transformed) of seed of two okra accessions dried to 6% SMC in drying room, prior to storage and after a 6-month storage period at 5°C and minus 15°C as affected by seed treatments, for two growing seasons. Germination was assessed under screenhouse conditions; plant survival (percentage data arcsine transformed) was assessed in the field at 3 weeks after transplanting.

Accession Country	Seed treatments	Germination after 6-month storage at 5°C 2013	Germination after 6-month storage at -15°C 2013	Plant survival in field 2013	Germination prior to storage 2014	Germination after 6-month storage at 5°C 2014	Germination after 6-month storage at -15°C 2014
VI046536 Thailand	FS[a]				46.70		
	T1[b]	25.72 b	13.91 b	21.83 b	64.62 ab	77.85 a	72.04 a
	T2	20.75 b	24.32 b	22.53 b	65.00 ab	71.78 a	66.71 a
	T3	69.38 a	77.03 a	73.20 a	49.11 b	72.04 a	65.90 a
	T4	19.17 b	26.33 b	22.75 b	75.69 a	69.64 a	65.57 a
	T5	20.35 b	18.91 b	19.63 b	67.50 ab	67.46 a	64.92 a
	T6	19.30 b	14.17 b	18.76 b	60.77 ab	64.52 a	70.47 a
VI050958 Zambia	FS[a]				77.30		
	T1[b]	71.30 a	68.55 a	27.55 b	84.06 a	76.01 a	81.26 ab
	T2	84.06 a	73.27 a	28.25 b	81.88 a	81.26 a	82.84 ab
	T3	73.06 a	75.05 a	59.14 a	69.86 ab	75.67 a	86.86 a
	T4	73.57 a	69.86 a	28.35 b	64.62 b	70.82 a	80.04 ab
	T5	82.84 a	65.75 a	26.07 b	84.06 a	78.81 a	70.69 b
	T6	82.84 a	74.84 a	25.62 b	82.84 a	84.06 a	82.84 ab

[a]FS = Germination of untreated fresh seed before drying
[b]Treatment means followed by different letters in a vertical column are significantly different (P<0.05) from each other, based on Tukey's HSD test.

followed by 24 hours soaking in water (T3) significantly enhanced germination of accession VI050476, prior to storage and after a 6-month storage period, under both storage conditions and during both years. Other seed treatments did not show a beneficial effect on germination.

While seed treatments did not enhance germination of VI054533 from Taiwan in 2013 at both storage environments as assessed under laboratory and screenhouse conditions, T3 significantly increased germination of this accession prior to storage and after a 6-month storage period at 5°C and -15°C, independent of assessment method—laboratory versus screenhouse (Tables 4 and 5). However, no significant differences in germination could be established for VI054533 from Taiwan after a 6-month storage period at minus 15°C when assessed under screenhouse conditions. Surprisingly, germination of untreated fresh seeds of both accessions—assessed before drying—was much higher than after drying in 2014 (Tables 4

and 5). When assessed under screenhouse conditions, germination of seeds of VI054533 from Taiwan was much lower after a 6-month storage at 5°C compared to germination prior to storage and after storage at -15°C, except for T3 (partial removal of seed coat) (Table 5).

Like the effect of T3 on seed germination of accession VI050476, this treatment also significantly enhanced survival rate of plants at three weeks after transplanting to the field. Although not beneficial for seed germination of VI050476, T4 (soaking in household vinegar for 2 hours) visibly enhanced survival rate of plants of accession VI050476 (landrace) after transplanting to the field (Table 5). All other treatments resulted in very poor stand establishment.

Accession VI054533 from Taiwan consistently showed superior germination under laboratory and screenhouse conditions, during both growing seasons, compared to VI050476 from Thailand, except for seed storage at 5°C in 2015 when assessed under screenhouse conditions, with almost identical germination percentages for both accessions. However, T3 was able to restore germination of VI054533 at this storage condition to similar or even higher levels measured prior to storage and after a 6-month storage period at -15°C (Table 5).

DISCUSSION

Okra

Effect of cultivar/genotype on germination and field establishment

Despite a lower 1000-seed weight, accession VI050958 from Zambia had a significantly better germination than VI046536 from Thailand, under both laboratory and screenhouse conditions and during both years of experimentation (Tables 1-3). This contrasts with results obtained by Sankar and Mani [19] who observed a positive correlation between 1000-seed weight and germination percentage of 10 okra cultivars. Survival of plants at three weeks after transplanting was similar among both accessions.

Accession VI046536 is a landrace collected from a home garden in Nongkhayang, Nongphai, Uthay Thani province, Thailand, hence did not undergo dedicated breeding. The breeding status of accession VI050958 is not stated in the WorldVeg database AVGRIS (http://seed.worldveg.org/search/passport). However, percentage germination and field performance in our trials would indicate the behavior of a commercial cultivar. Our findings are in line with other researchers who also reported an effect of genotype/cultivar on the germination of okra seeds [5,7]. A wide variation in the germination of different genotypes could also be deduced from individual feedback obtained from okra seed recipients served by the WorldVeg genebank (unpublished). Special attention should be given to landraces and semi-domesticated accessions, since these tend to have thicker seed coats than commercial varieties [12] resulting in poor germination response as observed in these trials.

Poor germination response of okra cultivars/genotypes might also have to do with SMC. It was observed that seed coat impermeability increases as seed moisture content decreases [12,20]. Similarly, other authors observed a strong positive correlation of SMC with germination and a strong negative correlation with hardseededness [5]. They reported hardly any effect on seed germination and hardseededness at 13% SMC, but a strong effect at 4-6% SMC, which is the standard SMC used by genebanks for long-term seed storage. Research conducted in Brazil indicated that angular pods have a relatively narrow safe harvesting period in the range of 45-55 DAA, while cylindrical pods have a longer harvesting period of 45-75 DAA without negative effect on germination and hardseededness [7]. This might be related with the seed moisture content which ranged from 11.5-13.3% at 45 DAA for angular pods and declined to 9.6-10.5% at 55 DAA, while seeds from round, cylindrical pods did not fall below 10% SMC, even at 75 DAA.

Table 4: Germination (percentage data arcsine transformed) of seed of two water spinach accessions dried to 6% SMC in drying room, prior to storage and after a 6-month storage period at 5°C and at minus 15°C as affected by seed treatments, for two growing seasons. Germination was assessed under laboratory conditions.

Accession Country	Seed treat-ments	Germination after 6-month storage at 5°C 2013	Germination after 6-month storage at -15°C 2013	Germination prior to storage 2014	Germination after 6-month storage at 5°C 2015	Germination after 6-month storage at -15°C 2015
VI050476 Thailand	FS[a]			46.00		
	T1[b]	9.94 b	3.08 b	27.06 bc	27.72 b	32.45 b
	T2	3.08 b	8.72 b	29.62 bc	28.64 b	28.97 b
	T3	64.92 a	67.46 a	76.89 a	89.67 a	83.11 a
	T4	8.72 b	3.08 b	33.80 bc	24.24 b	28.69 b
	T5	3.08 b	7.12 b	35.40 b	27.20 b	22.06 b
	T6	8.72 b	3.08 b	22.00 c	26.51 b	25.07 b
VI054533 Taiwan	FS[a]			81.00		
	T1[b]	73.92 a	77.03 a	58.82 b	65.27 ab	62.34 b
	T2	67.66 a	71.03 a	60.97 b	57.42 b	59.86 b
	T3	76.01 a	76.07 a	78.25 a	76.63 a	89.69 a
	T4	78.25 a	75.32 a	60.05 b	61.83 b	65.50 b
	T5	71.92 a	76.07 a	58.34 b	58.12 b	60.11 b
	T6	65.49 a	66.79 a	54.94 b	55.02 b	59.56 b

[a]FS = Germination of untreated fresh seed before drying

[b]Treatment means followed by different letters in a vertical column are significantly different (P<0.05) from each other, based on Tukey's HSD test.

Table 5: Germination (percentage data arcsine transformed) of seed of two water spinach accessions dried to 6% SMC in drying room, prior to storage and after a 6-month storage period at 5°C and minus 15°C as affected by seed treatments, for two growing seasons. Germination was assessed under screenhouse conditions; plant survival (percentage data arcsine transformed) was assessed in the field at 3 weeks after transplanting.

Accession Country	Seed treatments	Germination after 6-month storage at 5°C 2013	Germination after 6-month storage at -15°C 2013	Plant survival in field 2013	Germination prior to storage 2014	Germination after 6-month storage at 5°C 2015	Germination after 6-month storage at -15°C 2015
VI050476 Thailand	FS[a]				46.00		
	T1[b]	3.08 b	3.08 b	11.50 bc	27.06 bc	35.62 b	35.62 b
	T2	0.27 b	3.08 b	11.50 bc	29.62 bc	28.66 b	32.83 b
	T3	62.89 a	65.06 a	82.81 a	76.89 a	69.86 a	71.83 a
	T4	7.12 b	9.94 b	56.17 ab	33.80 bc	30.64 b	25.41 b
	T5	0.27 b	3.08 b	0.33 c	35.40 b	33.80 b	27.47 b
	T6	3.08 b	0.27 b	11.50 bc	22.00 c	26.28 b	27.56 b
VI054533 Taiwan	FS[a]				81.00		
	T1[b]	63.93 a	66.85 a	73.13 a	58.82 b	31.10 b	60.66 a
	T2	65.90 a	68.90 a	84.45 a	60.97 b	31.26 b	59.42 a
	T3	67.93 a	77.29 a	84.62 a	78.25 a	70.82 a	71.14 a
	T4	65.89 a	60.82 a	84.58 a	60.05 b	30.36 b	59.42 a
	T5	60.79 a	70.22 a	84.83 a	58.34 b	32.30 b	62.21 a
	T6	71.30 a	60.79 a	81.59 a	54.94 b	27.91 b	58.12 a

[a]FS = Germination of untreated fresh seed before drying

[b]Treatment means followed by different letters in a vertical column are significantly different (P<0.05) from each other, based on Tukey's HSD test.

VI046536 has angular-shaped pods and harvesting—done according to the maturity stage of the pods—ranged from 42-65 DAA, while VI050958 has round, cylindrical pods and harvesting ranged from 40-87 DAA. Other authors [8] also stressed the importance of pod harvesting at 40 DAA with later harvests leading to reduced seed germination. The extended harvest period of VI046536 with angular pods could perhaps partly explain the lower germination rate of this accession but further experimentation is needed to elucidate this aspect.

Effect of seed treatments on germination and field establishment

As okra seeds have been categorized as difficult to germinate due to physical dormancy [10], we conducted seed treatments prior to sowing with the aim to improve germination and field establishment. Partial removal of the seed coat followed by 24 hours soaking in water (T3) was beneficial for seed germination and plant survival in the field in 2013 (Tables 2 and 3). Given the relatively high percentage of germination of the control (T1) in 2014, seed treatments did not enhance germination during the second year. Differences in environmental conditions between the two years are unlikely to have contributed to this major change of the percentage of germination of the control plants in 2014, as the average monthly temperature was very similar during both years (data not shown). Moreover, germination in the screenhouse (Table 3) took place with regular daily watering of seedling trays until transplanting to the field, hence differences in the rainfall pattern, which were observed during both years (data not presented) are unlikely to have contributed to the change in the germination pattern between both years, unless seed conditioning happened during the seed filling stage already.

Hydropriming i.e. soaking okra seeds in water for 12 hours and solid matrix priming with calcium aluminum silicate for 24 hours significantly increased seed germination, seedling vigor, mean germination time and marketable fruit yield in okra cv. Hisar Unnat [6]. For ease of application, the researchers recommended hydropriming as a simple, economical and safe treatment to enhance seed germination and yield of okra. Our experiments do not confirm these results, as soaking seed in water for 24 hours (T2) did not have any significant effect on germination under laboratory and screenhouse conditions, and plant survival at three weeks after transplanting to the field, with both accessions and during both years of observation. Apparently, the seed coat poses a physical barrier to imbibition and scarification is needed to facilitate water uptake and enhance germination [10] and this has been confirmed in our experiments.

Effect of storage conditions on seed germination

We did not observe an effect of storage conditions (5°C–medium term and -15°C – long-term) on germination of both okra accessions, under laboratory and screenhouse conditions and during both years (Tables 2 and 3). Untreated seed of VI046536 showed enhanced germination after a 6-month storage period in both storage environments compared to germination prior to storage (Table 3). Hence long-term storage conditions as applied in genebanks to reduce regeneration intervals are safe regarding okra seed germination. Seed germination of the control (T1) of VI050958 tended to be lower under screenhouse conditions compared to laboratory conditions, while this trend could not be confirmed for VI046536 from Thailand. This effect might be due to the different substrate used in the screenhouse.

Water spinach

Effect of cultivar/genotype on germination and field establishment

In analogy to okra, the two water spinach accessions selected for the trials showed a contrasting germination response, independent of storage conditions and year of observation (Tables 4 and 5). The control (T1) of breeding line VI054533 from Taiwan with

a relatively high 1000-seed weight of 51.7 g consistently showed a high percentage of germination in the range of 80-90% under laboratory conditions and 76-80% under screenhouse conditions, while the control of accession VI050476 from Thailand with lower 1000-seed weight of 44.1 g showed poor germination in the range of 1-34% under both laboratory and screenhouse conditions, prior to storage and after a 6-month storage period. Similarly, survival of plants of VI054533 at three weeks after field transplanting was significantly superior to VI050476.

Germination of water spinach seeds is reportedly poor (<60%), especially after long storage periods at low SMC as is common practice in genebanks [11,12]. However, also for commercially traded seed, poor germination is a concern for breeding companies and high and uniform germination is a major breeding goal, especially when seeds are used for sprouting and microgreen production as is the case with water spinach in Asia [21]. With germination in the range of 80-90% as observed in our studies after low SMC storage, this aim has been achieved with VI054533 from Taiwan. However, when germination was assessed under screenhouse conditions (Table 5), performance of untreated seed (T1) of VI054533 was poor after storage at 5°C but was similar to the results obtained prior to storage when stored at minus 15°C. Germination of seeds exposed to treatment 3 (partial removal of seed coat) was not affected by the storage environment. In contrast to findings in the literature [11,12], our experiments showed that cold storage of water spinach seeds dried to low SMC levels is not problematic for germination. However, further experiments at longer storage intervals are needed to confirm this.

Effect of seed treatments on germination and field establishment

As poor germination of water spinach seeds has been attributed to hardseededness [11,12], we applied several seed treatments to improve germination and stand establishment. Partial removal of the seed coat followed by 24 hours soaking in water (T3) significantly enhanced germination of accession VI050476 as well as survival of plants at three weeks after field transplanting, under both storage conditions and both evaluation environments (laboratory and screenhouse) and during both years of experimentation (Tables 4 and 5). All other seed treatments did not enhance germination, a clear sign that the seed coat poses a physical barrier to imbibition. With accession VI054533 from Taiwan, seed treatments did not enhance germination during the first year but T3 again resulted in enhanced seed germination in 2015, under both storage conditions and evaluation environments (Tables 4 and 5). This is in line with observations made by other authors [22,23] who reported that mechanical scarification increased seed germination from 35% to 100% in several Ipomoea taxa.

Many leguminous species are also characterized by hardseededness and manual scarification has been shown to be the best way of stimulating seed germination [24], being superior to chemical scarification with sulphuric acid, which was only effective at high concentrations (70%) and with 60 minutes exposure time [13]. Household vinegar used in our experiments was either too weak or the exposure time was too short to result in any positive effect. As manual scarification is difficult to perform on large commercial seed lots, other mechanical abrasion mechanisms could be explored.

Effect of storage conditions on seed germination

In general, there was no obvious effect of storage conditions on seed germination of water spinach accessions (Tables 4 and 5). Surprisingly, germination of untreated seed (T1) of VI054533 was poor after storage at 5°C but was satisfactory and comparable to the results obtained prior to storage when stored at minus 15°C (assessment under screenhouse conditions). Seeds treated with T3 showed good performance under both storage conditions in 2015.

CONCLUSION

Our studies confirmed previous results that both okra and water spinach are difficult to germinate and to obtain good field establishment due to physical, seed coat-imposed dormancy. Moreover, significant genotypic variation among genebank entries/cultivars was found for both crops, making germination response difficult to predict. Improved varieties showed much better germination response than landraces and did not require seed treatments for satisfactory germination. Hydropriming, i.e. soaking of seed in water for 24 hours, did not have an impact on seed germination. Partial removal of the seed coat, followed by 24 hours soaking in water consistently resulted in high germination percentages of genotypes that had not undergone dedicated breeding (landraces) of both crops, under laboratory and screenhouse conditions and during two subsequent growing seasons. Mechanical seed abrasion mechanisms could be explored for seed lots that have not undergone breeding. When distributing seed samples of semi-domesticated accessions or landraces, genebanks should advise seed recipients that scarification methods may be necessary to obtain satisfactory seed germination and field establishment.

ACKNOWLEDGEMENT

Funding for this research was provided by the Federal Ministry for Economic Cooperation and Development, Germany and long-term strategic donors to the World Vegetable Center: Republic of China (Taiwan), UK aid from the UK government, United States Agency for International Development (USAID), Australian Centre for International Agricultural Research (ACIAR), Germany, Thailand, Philippines, Korea, and Japan.

REFERENCES

1. Ebert AW. Potential of underutilized traditional vegetables and legume crops to contribute to food and nutritional security, income and more sustainable production systems. Sustainability 2014;6:319-335.
2. Meldrum G, Padulosi S, Lochetti G, Robitaille R, Diulgheroff S. Issues and prospects for the sustainable use and conservation of cultivated vegetable diversity for more nutrition-sensitive agriculture. Agriculture 2018;8(7):112.
3. Schreinemachers P, Ebert AW, Wu M-H. Costing the ex situ conservation of plant genetic resources at AVRDC – The World Vegetable Center. Gen Res Crop Evol 2014;61(4):757-773.
4. De la Peña RC, Ebert AW, Gniffke P, Hanson P, Symonds RC. Genetic adjustment to changing climates: vegetables. In: Yadav SS, Redden RJ, Hatfield JL, Lotze-Campen H, Hall AE (eds.) *Crop Adaptation to Climate Change*, First Edition. John Wiley & Sons, Ltd., Chichester, West Sussex, UK, 2011;396-410.
5. El Balla MM, Saidahmed A, Makkawi M. Effect of moisture content and maturity on hardseededness and germination in okra (*Abelmoschus esculentus* L. Moench). Int J Plant Physiol Biochem 2011;3:102–107.
6. Sharma AD, Rathore SVS, Srinivasan K, Tyagi RK. Comparison of various seed priming methods for seed germination, seedling vigour and fruit yield in okra (*Abelmoschus esculentus* L. Moench). Scientia Horticulturae 2014;165:75–81.

7. Purquerio LFV, do Lago AA, Passos FA. Germination and hardseedness of seeds in okra elite lines. Hortic Bras 2010;28(2):232-235.

8. Mohammadi G, Khah EM, Ghitouli M, Shirkhani A, Shabani G. Improvement of okra (*Abelmoschus esculentus* L.) hardseedness by using micro elements fertilizer. Ann Biol Res 2012;3(5):2153-2161.

9. Doijode SD. Okra: *Abelmoschus esculentus* (L.) Moench. In: Seed Storage of Horticultural Crops, Food Products Press, New York. 2011;181-189.

10. Millennium Seed Bank (MSB). *Abelmoschus esculentus*.

11. Grubben GJH. *Ipomoea aquatica* Forsk. In: Grubben GJH, Denton OA (eds.) Plant Resources of Tropical Africa (eds.), Vegetables, PROTA Foundation, Wageningen, Netherlands; Backhuys Publishers, Leiden, Netherlands; CTA, Wageningen, Netherlands, 2004;2:332-335.

12. Ellis RH, Hong TD, Roberts EH. Handbook of seed technology for genebanks. Vol. I. Principles and methodology. Handbooks for Genebanks. International Board for Plant Genetic Resources, Rome, Italy. 1985a;2:210.

13. Patanè C, Gresta F. Germination of *Astragalus hamosus* and *Medicago orbicularis* as affected by seed-coat dormancy breaking techniques. J Arid Environ 2006;67:165–173.

14. ISTA. International Rules for Seed Testing. International Seed Testing Association. 2016;284.

15. Karrfalt RP. Equilibrium relative humidity as a tool to monitor seed moisture. In: Riley LE, Pinto JR, Dumroese RK (tech. cords.) National Proceedings: Forest and Conservation Nursery Associations-2009. Proc. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, CO, USA. RMRS 2010;62(62):45-47.

16. Royal Botanic Gardens Kew. Seed Information Database (SID), version 7.1. Seed Viability Equations - Estimating Seed Equilibrium. 2017.

17. Chachalis D, Korres N, Khah EM. Factors affecting seed germination and emergence of Venice mallow (*Hibiscus trionum*). Weed Sci 2008;56(4):509-515.

18. Ellis RH, Hong TD, Roberts EH. Handbook of seed technology for genebanks. Vol. II. Compendium of specific germination information and test recommendations. Handbooks for Genebanks. International Board for Plant Genetic Resources, Rome, Italy. 1985b;3:667.

19. Sankar YS, Mani A. Studies on Seed Quality Parameters of Okra (*Abelmoschus esculentus* L.). J Agric Technol 2015;2(1&2):79-83.

20. Demir I. Occurrence of hardseededness in relation to seed development in okra (*Abelmoschus esculentus* (L.) Moench). Plant Var Seeds 1997:10:7-13.

21. Ebert AW. High value specialty vegetable produce. In: Peter KV, Hzra P (eds.) *Handbook of Vegetables*, Volume 2, Studium Press LLC, Houston, USA. 2015;119-143.

22. Ogunwenmo KO. Variation in fruit and seed morphology, germination and seedling behaviour of some taxa of *Ipomoea* L. (Convolvulaceae). Feddes Repertorium: Z Bot Taxon Geobot 2006;117(4):207-216.

23. Ogunwenmo KO, Ugborogho RE. Effects of chemical and mechanical scarification on seed germination of five species of *Ipomea* L. (Convolvulaceae). Bol Soc Brot 2000;70:33-47.

24. Siles G, Torres JA, Ruiz-Valenzuela L, García-Fuentes A. Germination trials of annual autochthonous leguminous species of interest for planting as herbaceous cover in olive groves. Agric Ecosys Environ 2016;217:119–127.