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Morphological and Ecological Characteristics of Pointed Gourd (*Trichosanthes dioica* Roxb.)

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Pointed gourd (*Trichosanthes dioica* Roxb.) is a dioecious, fruit type cucurbit vegetable originated in India and Bangladesh. Immature fruits at green stage are commonly consumed as a vegetable after cooking. It is extensively cultivated during summer season in tropical and subtropical regions but potential to grow in temperate regions due to its wide range of phenotypic and genotypic variability. The exploitation of these variations might be the basis for subsequent improvement of this crop. The purpose of this study was to investigate morphological and ecological characteristics for the selection of suitable genotypes for cultivation in Japan. Mature vines of 33 (24 female and 9 male) pointed gourd accessions have been collected from Bangladesh and cultivated in glasshouse as well as open field at Hakozaki, Kyushu University in northern Kyushu, Japan. Among these genotypes PGF01, PGF02, PGF04, PGF08 and PGF17 have been selected for early flowering, maximum flesh content, fruit weight, fruit yield and fruit set rate, respectively. We also defined the germination potentiality of ripen seed treated with nineteen treatments. Scarification with conc. H_2SO_4 for 30 sec notably increased germination of 98.8% within two weeks though seeds soaked in tap water for 12 h provide 74.4% germination by a week. Therefore, tap water soaking for 12 h is considered as a cost effective, safe, time saving and convenient option for the farmers deal with large scale seed propagation of pointed gourd.

Key words: dioecious, fruit vegetable, pointed gourd, seed germination, *Trichosanthes dioica*.

INTRODUCTION

Pointed gourd (*Trichosanthes dioica* Roxb.) is a dioecious vegetable belongs to the cucurbitaceae family. This species is a climbing plant and originated in Indian subcontinent. It is one of the most important summer vegetables in India and Bangladesh where locally known as “Potol” or “Parwal”. Immature fruit which is 10~12 cm length and 30~50 g at green stage is the main edible part and rich in vitamin A, proteins, minerals and also claimed to lower total serum cholesterol and blood sugar (Pandit and Hazra, 2008 and Rai *et al*., 2008). Leaves and tuberous roots of this species are used in Ayruvedic medicine (Chandrasekar *et al*., 1989) and seeds for acid–dyspeptic disease treatment (Harit and Rathee, 1996).

Pointed gourd is usually grown in open field as trellis training under high temperature condition (30–35˚C) of its origin countries, but able to produce in temperate regions due to its adaptive potentiality (Singh and Whitehead, 1999). In India, it is extensively growin in ‘diara’ lands in North Bihar and in eastern Uttar Pradesh however in Assam, Bengal, Orissa etc., it is also grown in loamy soils and in places with hot and humid climate (Bose and Som, 1986). The main precaution is to prevent waterlogging. In Bangladesh, it is widely cultivated in the districts of Rajshahi, Bogra, Rangpur, Pabna, Jessore and Kustia (Rashid, 1993). Since this species is a vegetative propagated crop, there are several clones with distinct characters met with in different States. However, the morphological characteristics of pointed gourd under different ecological conditions than that of their origins have never been investigated apart from Singh and Whitehead (1999) report.

Selection of suitable genotypes with desired traits would be very essential for the improvement of this crop. However, seed germination is poor in pointed gourd (Bose and Som, 1986). Thus, lack of the information of seed germination technique in pointed gourd is a bottleneck for genetic improvement studies. Therefore, a standard seed germination protocol would be helpful to provide a rational contribution for the advancement breeding efforts of pointed gourd.

The present investigation was carried out at glasshouse and open field to select a suitable genotype in response to the morphological and ecological characteristics and propose an efficient seed germination technique for large scale multiplication of pointed gourd species.

MATERIALS AND METHODS

Plant sample collection and cultivation

Mature vine cuttings of 24 female and 9 male genotypes of pointed gourd were collected from different locations of Bangladesh. Among the collected vines, 14 female genotypes and 4 male genotypes were planted in the 30 cm diameter plastic pot with 1:1 (pamis sand : akadama soil) growing mixture and placed in the unheated glasshouse at Hakozaki campus (lat. 33° 37’ N; long. 130° 25’ E), Kyushu University. Remaining 10 female and 5 male genotypes were planted in the

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experimental open field of the same university at a spacing of 2 × 1 m. Female accessions were denoted as PGF01~PGF24 and PGM01~PGM09 for male. Only three emerged shoots per accession were grown after germination of respective vine cuttings to further growth those considered as three replications.

Morphological and ecological characteristics

The accessions were recorded for different morphological and ecological characters such as days to the first flowering from planting, number of flower (male and female), number of fruits, fruit length and fruit weight in both of their growing habitats. Hand pollination was done during night at anthesis and 5 fruits at green edible stage of each female accession were harvested and fruit length, fruit weight were measured. Fruit set rate was calculated with the following formula: fruit set rate (%) = (number of fruit obtained / number of pollinated flowers) × 100.

Seed germination test

Fully mature and ripen fruits (55–60 days after anthesis) of pointed gourd were harvested from plants grown in the glasshouse and open field in Hakozaki campus, Kyushu University. The extracted seeds were washed with running tap water to fully removal of placental hairs. Moreover, seeds were still covered with mucilaginous layer at outside of the seed coat. To remove this layer, seeds were gently scrubbed on the rough surface of steel made sieve without causing external injury in the seed coat. Thoroughly cleaned seeds were spread onto paper towels and kept at room temperature to dry. The dry seeds kept on a plastic zip lock bag and stored in a freezer at 4˚C until use for germination.

For germination test, thirty seeds were used for each treatment and primed with nineteen (19) treatments. Physical scarification of the seeds was done by shaking in a plastic bottle with quartz sand for 30 and 60 sec, tap water soaking for 6, 12 and 24 hours, hot water (HW) treatment at 60, 80, 100˚C temperature for 10, 30, 60 sec. Concentrated H_2SO_4 soaking for 10, 30 and 60 sec was used as chemical scarification techniques. Gibberellic acid (GA_3) at 100 ppm for 24 hours soaking was taken as hormone regulation treatment. No treatments were applied in case of control sample seeds. After applying the treatments, seeds were rinsed in running tap water and put on paper towels at room temperature to air dry. The treated seeds were soaked in GA_3 solution (100 ppm) for 24 hours excluding control and water treated samples. After 24 hours, seeds were rinsed properly with running tap water and placed on 90 mm diameter petri dishes with two layers of filter paper (ADVANTEC No. 2) which were moistened with 5 ml fungicide solution (Benlate 1000 ppm) to prevent fungal contamination. All petri dishes were placed in incubator (SANYO Incubator, MIR–154, Japan) at 30˚C in dark condition. Germination was defined as emergence of the radicle through the seed coat (≥ 1 mm) (International Seed Testing Association ISTA, 1999). Germinated seeds were counted daily and continued for 30 days. The first day of germination from seed sowing, last day of germination from seed sowing, germinating period and final germination percentage was calculated. The test was replicated for three times. Each replication contains 19 treatments and 30 seeds were used for each treatment.

RESULTS

Morphological and ecological characteristics of pointed gourd

Morphological characteristics of 14 female pointed gourd accessions cultivated in glasshouse are shown in Table 1. The flower which appeared earliest was in PGF01 and 76 days required after planting whereas it required more than 90 days in PGF03, 04, 05 and 10. Other remaining accessions were produced first flower within 80~90 days after planting. More than 80% of fruit set rate (%) were observed in PGF06 (83.8%) and 08 (88.4%), though PGF01 showed the lowest fruit set rate (37.7%). Remarkable variations were observed among the accessions according to fruit length. The longest fruit (13.3 cm) was produced by PGF04 followed by PGF03 (12.5 cm) and PGF01 produced the shortest (7.5 cm). Although, there was no significant variation observed for fruit diameter (data not shown), the fruit more than 55 g were obtained in PGF03, 04, 08 and 12 which had relatively long shape fruits.

On the other hand, morphological and ecological characteristics of 10 pointed gourd accessions cultivated in the open field are shown in Table 2. Early flowering was noticed in PGF19, which took comparatively short time 109.0 days then others and statistically similar results were observed in PGF18 and PGF17. Meanwhile, delay flowering was found in PGF21 (132.3 days) followed by PGF22 (131.3 days) and PGF23 (131.0 days). 44.5% fruit set rate was found in PGF24 while more than two times higher of it fruit set was produced by PGF17 (92%). The highest fruit length (13.0 cm) was appeared in PGF24 whereas PGF15 and PGF19 produced the shortest (8.5 cm) compared to other accessions produced fruits. Individual fruit weight was also distinguished from each other, which ranged from 36.4 g (PGF23) to 58.3 g (PGF22).

Flesh content and fruit yield

The highest flesh content (19.9) was measured in PGF02 followed closely by that of PGF03, PGF05 and the lowest was in PGF06 (6.4) in the glasshouse (Fig. 1). Similarly, the maximum flesh content (19.1) was observed in PGF24 produced fruit and the minimum (8.4) was in PGF17, which was followed closely by that of PGF22 in open field (Fig. 2).

The fruit yield per plant exceeded 1000 g/plant for PGF08, PGF11 and PGF07 in the glasshouse (Fig. 3). In particular, PGF08 accession produced the highest yield, with 1695 g/plant. In contrast, PGF14, PGF03, PGF01 and PGF05 had the yield of less than 500 g/plant and PGF05 had markedly the lowest yield of 241 g/plant. Meanwhile, similar tendencies were also observed in

Table 1. Characteristics of 14 pointed gourd accessions cultivated in glasshouse

Accession	Days to first flowering (day)	Fruit set rate [z] (%)	Number of fruit/plant	Fruit length (cm)	Fruit weight (g)
PGF01	76.0 e [y]	37.7 g	6.3 h	7.5 h	42.9 f
PGF02	88.0 bcd	58.3 ef	10.3 fg	12.0 bc	52.1 c
PGF03	91.6 ab	68.6 cde	6.7 h	12.5 ab	57.7 ab
PGF04	94.3 a	73.0 bcd	9.7 g	13.3 a	60.4 a
PGF05	95.6 a	78.2 abc	4.7 h	9.5 ef	51.7 c
PGF06	87.0 cd	83.8 ab	20.6 c	10.6 d	39.0 g
PGF07	86.0 cd	71.9 bcd	23.0 b	9.4 f	47.6 de
PGF08	88.0 bcd	88.4 a	30.3 a	11.5 c	55.9 b
PGF09	87.0 cd	68.8 cde	17.0 d	10.1 def	44.8 ef
PGF10	95.0 a	70.9 bcde	14.6 e	10.5 d	37.8 g
PGF11	87.6 bcd	71.4 bcde	28.3 a	10.2 de	50.6 c
PGF12	83.6 d	53.6 f	12.0 f	11.4 c	58.9 a
PGF13	86.6 cd	62.4 def	11.6 fg	10.2 de	49.2 cd
PGF14	89.0 bc	60.0 def	12.0 f	8.5 g	39.5 g

[z] Fruit set rate (%) = (Number of fruit/Total number of pollinated flowers) × 100.
[y] Means followed by the different letters within a column are significantly different ($P < 0.05$) by Honestly significant difference test using R–software.

Table 2. Characteristics of 10 pointed gourd accessions cultivated in open field

Accession	Days to first flowering (day)	Fruit set rate [z] (%)	Number of fruit/plant	Fruit length (cm)	Fruit weight (g)
PGF15	120.0 c [y]	55.7 cd	4.7 e	8.5 f	46.8 c
PGF16	116.0 cd	64.4 bcd	2.3 f	11.5 c	38.9 f
PGF17	112.3 de	92.0 a	11.6 b	12.0 b	41.0 e
PGF18	109.3 e	80.0 ab	13.3 a	9.3 e	39.5 ef
PGF19	109.0 e	68.2 bc	5.0 e	8.5 f	54.9 b
PGF20	126.7 b	55.7 cd	3.0 f	12.2 b	41.0 e
PGF21	132.3 a	69.7 bc	4.3 e	11.4 c	44.6 d
PGF22	131.3 ab	66.5 bc	10.0 c	9.2 e	58.3 a
PGF23	131.0 ab	81.2 ab	11.6 b	10.3 d	36.4 g
PGF24	116.0 cd	44.5 d	7.0 d	13.0 a	48.3 c

[z] Fruit set rate (%) = (Number of fruit/Total number of pollinated flowers) × 100.
[y] Means followed by the different letters within a column are significantly different ($P < 0.05$) by Honestly significant difference test using R–software.

open field (Fig. 4) where PGF22 and PGF18 accessions were produced 584.0 and 527.8 g fruit/plant, respectively. However, other accessions were produced less than of that amount and notably PGF16 had produced only 90.9 g fruit yield per plant.

Seed germination test

The highest germination percentage (98.8%) was obtained from seeds scarified with con. H_2SO_4 for 30 sec (Fig. 5). The germination percentage was more than 70% when seeds were treated with water soaking for 12 to 24 h and hot water soaking at 60 and 80°C for 60 and 10 sec. Specifically, 74.4% seeds were germinated with the treatment of immersing of seeds in tap water for 12 h, which was identical with hot water at 80°C for 10 sec treatment. There was no germination for the hot water at 100°C for 60 sec and control treated seed samples.

The response of seeds to germinate and how quickly they would be germinated was significantly differing based on the pre–sowing treatments (Table 3). However, seeds of pointed gourd were started to germination within 5–10 days after sowing, for most of the pretreatments but sand quartz shaking for 60 sec, tap water soaking for 12 h and H_2SO_4 scarification for 30 sec treated seeds were displayed exception to take less than 5 days to first response of germination. In addition, about 3 weeks were required to complete the whole germination process for most of the treated seeds, except for tap water soaking for 6 h, 12 h and hot water soaking at 100°C for 30sec where they only took less than 1 week to complete.

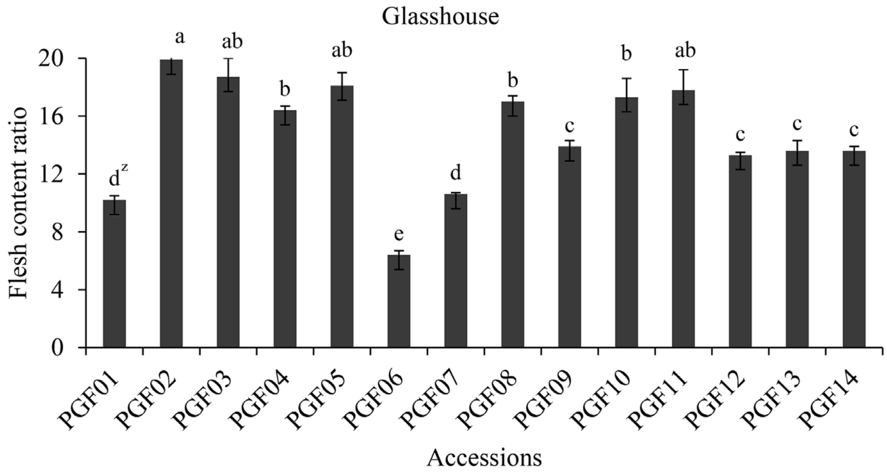

Fig. 1. Flesh content ratio of 14 pointed gourd accessions cultivated in glasshouse.
[z] Different letters indicate significantly different ($P < 0.05$) by Honestly significance difference test. Bars represent mean ± SE.

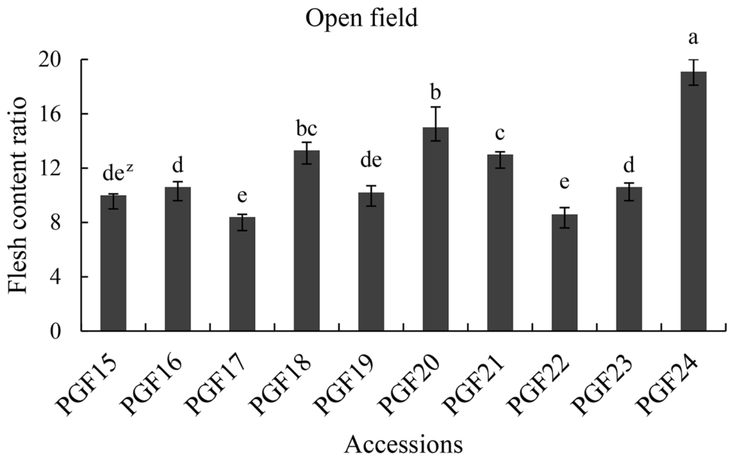

Fig. 2. Flesh content ratio of 10 pointed gourd accessions cultivated in open field.
[z] Different letters indicate significantly different ($P < 0.05$) by Honestly significance difference test. Bars represent mean ± SE.

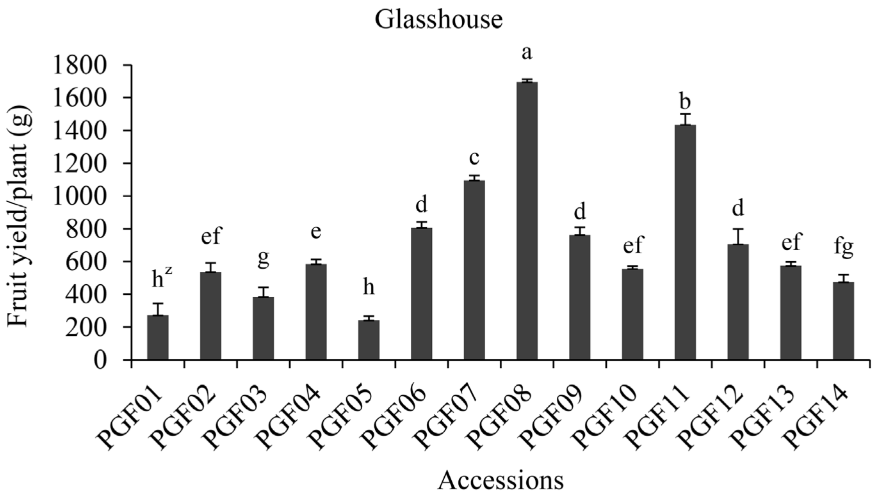

Fig. 3. Fruit yield/plant (g) of 14 pointed gourd accessions cultivated in glasshouse.
[z] Different letters indicate significantly different ($P < 0.05$) by Honestly significance difference test. Bars represent mean ± SE.

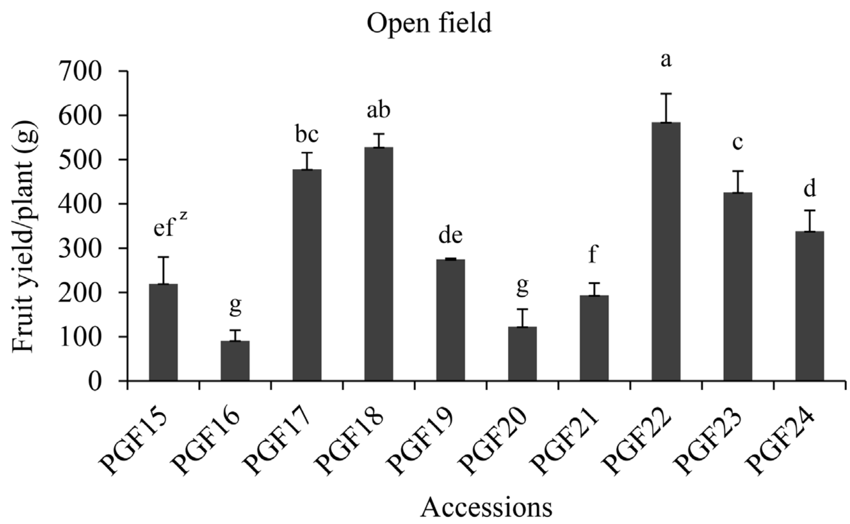

Fig. 4. Fruit yield/plant (g) of 10 pointed gourd accessions cultivated in open field.
[z] Different letters indicate significantly different (P < 0.05) by Honestly significance difference test. Bars represent mean ± SE.

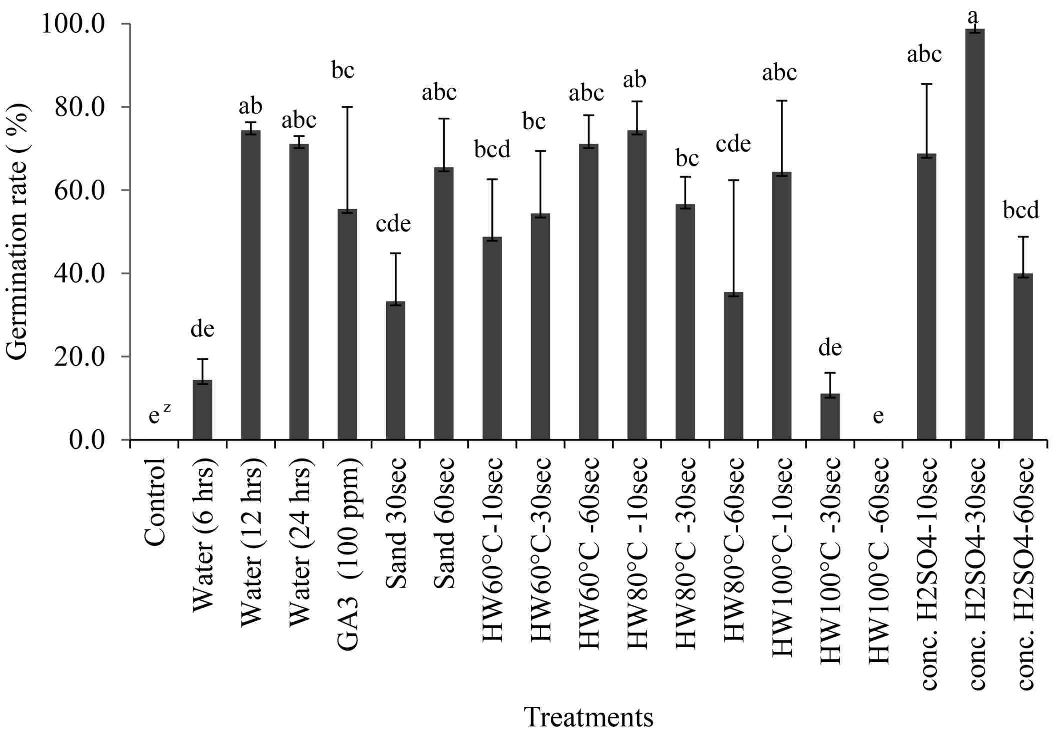

Fig. 5. Treatments effect on pointed gourd seed germination.
Sand: Quartz sand, HW: Hot water
[z] Different letters indicate significantly different (P < 0.05) by Honestly significance difference test. Bars represent mean ± SE.

Table 3. Treatments effect on germination timing attributes of pointed gourd seed

Treatments [z]	First day of germination from seed sowing (day)	Last day of germination from seed sowing (day)	Germinating period (day)
Control (without treat.)	– [y]	–	–
Water (6 h soaking)	7.0 ab [x]	8.0 c	1.0 c
Water (12 h soaking)	4.0 b	8.0 c	4.0 c
Water (24 h soaking)	7.3 ab	26.0 ab	18.6 ab
GA_3 (100 ppm 24 h soaking)	6.6 ab	26.0 ab	19.3 ab
Sand 30 sec+GA_3	5.0 ab	24.6 ab	19.6 ab
Sand 60 sec+GA_3	3.6 b	24.6 ab	21.0 a
HW60°C –10 sec+GA_3	10.0 ab	29.0 a	19.0 ab
HW60°C –30 sec+GA_3	8.6 ab	29.3 a	20.6 ab
HW60°C –60 sec+GA_3	5.6 ab	25.3 ab	19.6 ab
HW80°C –10 sec+GA_3	8.3 ab	29.6 a	21.3 a
HW80°C –30 sec+GA_3	6.3 ab	26.3 ab	20.0 ab
HW80°C –60 sec+GA_3	10.6 ab	20.6 abc	10.0 bc
HW100°C –10 sec+GA_3	6.0 ab	21.3 ab	15.3 ab
HW100°C –30 sec+GA_3	12.3 a	15.3 bc	3.0 c
HW100°C –60 sec+GA_3	–	–	–
conc. H_2SO_4 –10 sec+GA_3	8.3 ab	23.6 ab	15.3 ab
conc. H_2SO_4 –30 sec+GA_3	4.3 b	19.6 abc	15.3 ab
conc. H_2SO_4 –60 sec+GA_3	6.6 ab	27.0 ab	20.3 ab

[z] All seeds were soaked in GA_3 solution (100 ppm) for 24 h after each treatment except control and water treatments. Sand: Quartz sand, HW: Hot water.
[y] No seed germination.
[x] Means followed by the different letters within a column are significantly different ($P < 0.05$) by Honestly significant difference test using R-software.

The results also indicated that, among 19 pretreatments investigated, tap water for 12 h, hot water at 80°C for 10 sec and H_2SO_4 scarification for 30 sec were found as effective to provide maximum germinated seeds than others. But the response of these seeds was varied due to the pretreatment differences. Seeds treated with tap water soaking for 12 h had faster response by 4 days and less than one week required to complete germination, which was followed closely by H_2SO_4 scarification for 30 sec to response while it took two weeks to complete. Besides, first germination response was the slowest and two times higher days (8.3) were required for seeds treated with hot water soaking at 80°C for 10 sec compared with the aforementioned treatments. In addition, it required more than three weeks (21.3 days) to compete the germination process.

DISCUSSION

Morphological and ecological characteristics

The variation observed for all the studied morphological, ecological and yield attributes was significant among the evaluated accessions in glasshouse and open field cultivation and this finding agrees with the earlier findings (Sharma *et al.*, 1988). El–Hamed and Elwan (2011) stated that the higher the proportion of the phenotypic variation attributed to the genotypic differences, the greater the feasibility of genetic manipulation to improve crop performance. Similar trends were also portrayed in our present findings where there was no consistency observed among the investigated accessions for all the evaluated characters at both glasshouse and open filed. Jena *et al.* (2017) observed fruit length range from 5.1–11.2 cm, fruit weight 20.0–42.0 g, flesh content 4.5–17.9 among 22 genotypes of pointed gourd evaluated in India and 8.1–11.9 cm, 24.3–56.3 g, 7.7–20.3 among 24 genotypes evaluated in Bangladesh by Kabir (2007). The average fruit length ranges from 7.5–13.3 cm, individual fruit weight 37.8–60.4 g, flesh content 6.4–19.9 at glasshouse (Table 1, Fig. 1) and 9.2–13 cm, 41.0–58.3 g, 8.4–19.1 at open field (Table 2, Fig. 2) was obtained in the present study. It clearly demonstrated that pointed gourd accessions were successfully adapted in northern Kyushu, Japan. Razim (2011) claimed that the accessions may exhibit superior yield in one location or environment, but this may not be constant in other environment with different agro–ecologies because the performance of a genotype mainly depends on environmental interaction. Wide differences between phenotypic and genotypic variation were indicating their sensitiveness to environmental fluctuations whereas narrow difference showed less environmental interference on the expression of these traits (Jena *et al.*, 2017). Our present study findings are mostly concerned about the phenotypic variability however; further precise investigation should be made to find out the environment influence on flowering habit, fruit setting and yield at both glasshouse and open field condition.

Seed germination test

There is no scientific information on the seed propagation of pointed gourd apart from Kumar *et al*. (2008), where they concluded that mature seeds failed to germinate scarified with 1N HCl for 15–30 min or 1N H_2SO_4 for 15 min. Meanwhile, this observation contradicts with our present findings, where 98.8% seeds were germinated scarified with conc. H_2SO_4 for 30 sec. It indicates that H_2SO_4 may have additional effects on seed coat chemistry to cracks, which permit water and gases into the seed resulting in enzymatic hydrolysis and thus transforming the embryo into a seedling (Wada *et al*., 2011). Besides, we also noticed 74.4% germination when seeds soaked in tap water for 12 h and hot water at 80°C for 10 sec. The possible fact is that it may be stimulates series of biochemical change in the seed that are essential to initiate the emergence process like softening seed–coat, hydrolysis, metabolism of growth inhibitors, imbibition, activation of enzymes (Ajouri *et al*., 2004). Surprisingly, in our study 55.5 and 65.5% seeds were germinated with GA_3 (100 ppm for 24 h soaking) and sand quartz shaking (60 sec) treatments those are lower than that of water soaking and H_2SO_4 scarification. These results indicate that pointed gourd seed has neither physical imposed dormancy by the testa nor physiological dormancy. Similar results were also observed in *Artemisia herba–alba Asso* seed germination process (Bakali, 2015).

Moreover, conc. H_2SO_4 scarified for 30 sec treated seeds were reached their maximal germination at 15.3 days after sowing followed by water soaking technique for 12 h while both treated samples were responded faster at 4 days after sowing. On the contrary, hot water at 80°C for 10 sec treated seeds were provided the similar amount of germination but it took maximum time to first response as well as completion of whole germination process. Based on the possibility of improving the germination and acceleration capabilities of this process, tap water soaking for 12 h and conc. H_2SO_4 scarification for 30 sec can be selected as the best suited techniques for pointed gourd seed germination. It has been reported that acid scarification, is commonly not preferred due to its cost, safety risk, environmental precautions and operationally not applicable by the farmers deal with large seed lots (Chavez *et al*., 2010). From these findings, seed soaking in tap water for 12 h would be the most effective and convenient option compared to others.

In conclusion, it can be stated that though pointed gourd belongs to tropical and subtropical regions crop, but it is successfully cultivated in glasshouse and open field condition in northern Kyushu of Japan. Among the investigated accessions, PGF01, PGF02, PGF04, PGF08 and PGF17 can be selected regarding early flowering, maximum flesh content, fruit weight, fruit yield and fruit set rate respectively for further improvement of this crop. Soaking in tap water for 12 h has been proposed as a cost effective and safe technique for large scale seed germination of pointed gourd.

AUTHOR CONTRIBUTIONS

Jahidul HASSAN, conceived the idea of the study, designed experiment, performed the research and data analysis, and wrote the manuscript. Ikuo MIYAJIMA, supervised the work, provided suggestions and comments on the manuscript. Both authors assisted in editing the manuscript and approved the final version.

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