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The Effect of Seed Extraction Methods on Seed Quality of Two Cultivar's Tomato (*Solanum lycopersicum* L.)

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Abstract: In order to compare of different methods and identify the optimum condition for tomato seed extraction, factorial experiments with 3 replications was conducted. In the first experiment, pulp of two tomato cultivars (Faraon, Dominator) were fermented at two temperatures (25, 35°C) and six periods (24, 48, 72, 96, 120, 144 h). The germination of seeds in laboratory as well as seedling emergence and preliminary growth in greenhouse were studied and measured. The results showed that effect of cultivar on traits (except of seedling emergence) was significant. Also the effect of temperature of fermentation, duration of fermentation and also interaction effects of them on seed germination were significant. Totally seed quality decreased with increasing temperature and duration of fermentation and the fermentation duration from 24 to 48 h at temperature 25°C, is recommended. In the second experiment, tomato seeds were extracted by HCL (pH was arranged to 1, 2, 3 for 10, 20, 30 min), H_2SO_4 (pH was arranged to 1, 2, for 15, 30 min), Sodium carbonate (5, 10% for 24 and 48 h) and fermentation. Percentage germination, germination rate, length of radicle and length of plumule were used for seed quality assessment. The results showed that interaction effect between pH and duration of HCL treatments was significant for seed germination (percentage and rate) and there was an interaction effect between concentration and duration for germination rate in alkali treatments. Different extraction methods had not detrimental effect on percentage germination, but acid treatments produce very bright clean seeds in compare to other treatments.

Key words: Fermentation, temperature, period, acid, sodium carbonate

INTRODUCTION

The seed quality of tomato is affected by factors such as seed extraction method, duration and fermentation temperature for seed extraction and fruit maturity (Kailappan and Karunanithy, 2006). Tomato seed extraction involves a treatment to remove the gelatinous coating around the seeds. Processing of tomato seeds that includes several steps is accomplished by pulping by machine or hand followed by removal of the gel surrounding the seeds by fermentation, chemicals or by mechanical means (Silva *et al*., 1982). Advantages and disadvantages of each technique depends on temperature, application period and concentration (Demir and Samit, 2001).

In fermentation methods, the selected ripe fruits are harvested, crushed and allowed for fermentation in non-metallic containers at room temperature for two to three days. The pulps remain in the extract until the gel surrounding the seeds has been degraded by microorganisms (Eevera and Vanangamudi, 2006). The mixture should be stirred at once or twice a day to maintain a uniform rate of fermentation, also to release the seeds entrapped in the floating pulp and to prevent a fungal growth from starting at the surface of the mass, which can injure or discolour seeds (Raymond, 1999). When fermentation is complete, the good seeds settle down at the bottom and much of the pulp floats at the top, leaving a layer of clear liquid in between (Desai, 2004). Fermentation should take place long enough for the sufficient disintegration mucilaginous material adhering to the seed. The speed or duration of fermentation process is largely dependent on the temperature. If it remains between 24 to 27°C, satisfactory separation of seed and pulp may be achieved within 48 to 72 h (Agrawal, 1995). In relatively high temperatures (25-30°C) the fermentation is complete in approximately 24 h (Fenwick-Kelly and George, 1998). Excessive fermentation reduces seed quality. Usually the longer the fermentation time and the higher the temperature resulted in the greater reduction in germination (Silva *et al*., 1982). Fermentation should be a controlled process because if continued too long, it

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creates heat and mechanical injury to the seeds (McDonald and Copeland, 1997). Also long fermentation can cause premature sprouting (Eevera and Vanangamudi, 2006). Liptay (1989) stated that with long term fermentation percentage germination was declined severely at 25°C or higher temperatures.

Acid extraction methods especially hydrochloric acid is used largely for separating tomato pulp and seeds (Silva *et al*., 1982). This method is often favoured by large commercial producers as it rapidly degrades the gelatinous seed coating resulting in the production of a very bright clean seed sample (Gowda *et al*., 1991). Efficient breakdown of the gel surrounding the seed and quick cleaning, avoiding of the low and high temperatures problem, eradication of bacterial canker and producing bright looking seed coat are important features of acid extraction method (Desai, 2004). However, it can be deteriorative on seed quality if application period and concentration are not appropriate. Same concentration of sulphuric acid and three percent nitric acid could also be used instead of hydrochloric acid (Singh *et al*., 1985). If sulphuric acid added to the pulp without dilution was very corrosive and it damaged the seed (Kailappan and Karunanithy, 2006). Sodium carbonate method darkens the seed coats and is, therefore not normally used for commercial seeds (McDonald and Copeland, 1997).

Brar and Singh (1984) observed that different extraction methods did not significantly affect on seed recovery, but Yadav *et al*. (2004) reported that none of the extraction methods was detrimental to germination, but the seed recovery in fermentation method was highest. Some investigators reported that the best quality seed is obtained by fermentation method, because it is a natural process that is least harmful to the seed and can eradicate bacterial canker and other seed borne diseases (Das *et al*., 1997; Olympio and Dankyir, 1999). While Silva *et al*. (1982) and also Jadli and Singh (2009) found that natural fermentation resulted in poor breakdown of the gel surrounding the seed and significantly reduced seed vigour, so is not suitable method to obtain maximum seed quality in tomato. Vadivelu and Srimathi (1988) reported that the highest seed quality, as defined by percentage germination and seed recovery observed for acid extraction method and lowest was recorded in fermentation method. Gowda *et al*. (1991), Ghosh and Syamal (1997) Jadli and Singh (2009) stated acid treatments, alkali treatment, hydrochloric acid and alkali treatment as the best extraction method, respectively.

Supplying of high quality seeds is the basic requirement and it contributes greatly to the success any crop. Because of the importance of tomato, large quantities of high quality tomato seeds are required worldwide. In Iran, factors like inadequate seed production technology, absence of suitable varieties and poor post harvest seed handling resulted in the unavailability of good quality seed became a major problem. Then, two experiments were conducted to identify the optimum condition for fermentation treatment and also compare the effect of different seed extraction procedures on the quality of the seed obtained from tomato.

MATERIALS AND METHODS

Tomato (*Solanum lycopersicum* L.) plants of Faraon and Dominator cultivars were grown at the agricultural research glasshouse, Ferdowsi University of Mashhad during 2007-2008. Pulps were removed from fruits with uniform size and maturity.

In the first experiment the pulp of two tomato cultivars were fermented in glass beakers in incubators set at 25 and 35°C for each of the following durations: 24, 48, 72, 96, 120 and 144 h. The slurry was stirred twice daily during fermentation. At the end of treatments seeds were washed in running tap water, cleaned, surface dried on blotting paper and left on top of filter paper to dry at room temperature for 3 days. Final seed moisture content was determined by the high temperature oven method (ISTA, 1985) after drying and it was adjusted to <10%. Then seeds were evaluated in the laboratory at 25-30°C using the percentage germination and germination rate traits. The seeds were surface sterilized with 5% of sodium hypo-chlorite for 5 min and then washed with distilled water three times. Germination tests of both cultivars were done on three replicates of 30 seeds of every period at temperature 25 and 35°C in 9.0 cm diameter petri dishes with blotting paper for 14 days. In order to keep the papers moist, but avoiding free water, two sheets of paper were used with 5 mL of water and then added as required during the test. Germination was considered to have occurred when the radicle was at least 2 mm long. Germinating seeds were counted daily for 14 days and counts were made every day at the same time during the test. Seedling emergence and preliminary growth tests carried out in a glasshouse. Three replicates of 20 seeds from each of the 24 lots (2 cultivars × 2 temperatures × 6 periods) were sown in seedling trays. The seedling emergence (appearance of the hook at the surface), height and the number of leaf/seedling were monitored and measured after 28 days.

In the second experiment seed extraction techniques used were acid extraction by soaking in commercial hydrochloric acid HC1 (37%), H_2SO_4 (98%), alkali extraction by soaking in Sodium carbonate and natural

fermentation for 48 h at ambient temperature for both cultivars. Pulp of fruits was fermented 48 h at room temperature for natural fermentation and pH was arranged to 1, 2, 3 by adding required HCL to the slurry and kept at room temperature for 10, 20 and 30 min for HCL extraction. Sulphuric acid treatment, was done by using H_2SO_4 (98%) for arranging slurry to pH 1, 2 and then kept for 15 and 30 min at room temperature. During acid treatments the slurry was stirred with a magnetic stirrer. For alkali extraction method the pulp containing the extracted seeds was mixed with equal volume of 5 and 10% solutions of sodium carbonate and then mixtures were left 24 and 48 h at room temperature. After each method time the seeds mixture were washed with distilled water 4-5 times and subsequently dried in the same manner as in the natural fermentation method. Percentage germination, germination rate, length of radicle and length of plumule were used for seed quality assessment. Petri dishes were tightly closed by wax film to prevent possible evaporation and length of radicle and plumule was recorded after 14 days. The studies were conducted in factorial experiments in a completely randomized design with three replications.

Finally different extraction methods in Faraon cultivar were compared. This experiment was carried out in three replications and the data were analyzed in completely randomized design.

Duncan's multiple range tests was used for comparing the means of traits. Treatments were compared by applying Least Significant Difference (LSD) test at a 5% level of significance.

RESULTS AND DISCUSSION

The effect of temperature and duration of fermentation: Results in Table 1 indicated that significant differences were found on traits (except of seedling emergence) among two cultivars. Seeds extracted from cultivar Faraon had significantly higher mean than those extracted from the cultivar Dominator. Similar to our finding, Brar and Singh (1984), Singh *et al.* (1985) and Valdes and Gray (1998) reported that the cultivar had a significantly effect on some seed quality traits. The effect of temperature of fermentation and duration of fermentation on all traits was significant, so that increasing duration and temperature during the fermentation drastically reduces the seed germination and other seed quality traits including seedling emergence, height and the number of leaf/seedling (Table 1). The best results were obtained from 24-48 h fermentation. This agrees with Singh *et al.* (1985), Liptay (1989), Pandita *et al.* (1996) and Vishwanath *et al.* (2006). Whereas Baldo and Vallador (1985) and Kanwar (1989) found that eight and three days of natural fermentation was the best for getting good quality tomato seed, respectively. Also, Reyes *et al.* (2007) reported that increasing the fermentation time up to 7 days had not significantly effect on germination, but seed vigour decreased with fermentation longer than 5 days. These differences can be due to genotype, environment, fermentation condition and various seed quality tests.

McDonald and Copeland (1997) stated that prolonged fermentation affected the seed germination, because it creates heat and cause mechanical injury to the seeds. Also Eevera and Vanangamudi (2006) found that long fermentation can cause premature sprouting and therefore reduce total germination and seed quality.

Interaction effects were significant for most of the seed quality traits. As shown in Fig. 1a and d, in both cultivars seed germination decreased significantly with increasing temperature from 25 to 35°C. Cultivar Faraon at temperature 25°C resulted in the highest percentage germination (89.6%) and germination rate (7.1 seed/day). Also seed germination decreased with increasing period from 24 to 144 h (Fig. 1b, e). Cultivar Faraon for 24 h attained maximum percentage germination and germination rate with 92.78% and 9.0 seed/day, respectively and the lowest mean as 33.9% and 1.5 seed/day was recorded in cultivar Dominator for 144 h. In all fermentation periods, the Dominator cultivar

Table 1: The effect of temperature and duration of fermentation on seed quality of two cultivar's tomato

Traits	Germination (%)	Germination rate (seed day^{-1})	Seedling emergence	Height of seedling	No. of leaf/seedling
Cultivar					
Faraon	68.3a	4.8a	66.9a	5.8a	4.7a
Dominator	61.3b	4.2b	63.5a	5.2b	4.2b
Temperature (°C)					
25	88.0a	6.7a	86.2a	6.8a	5.3a
35	41.7b	2.3b	44.2b	4.2b	3.6b
Time (h)					
24	91.4a	7.9a	92.9a	7.3a	5.4a
48	85.0a	6.6b	85.0a	7.3a	5.5a
72	71.7b	4.4c	72.5b	5.7b	5.1a
96	56.1c	3.5d	55.4c	5.5b	4.7a
120	45.8d	2.7e	44.2d	4.0c	3.2b
144	38.9e	1.8f	41.2d	3.3c	2.7b

Values with different are significantly different

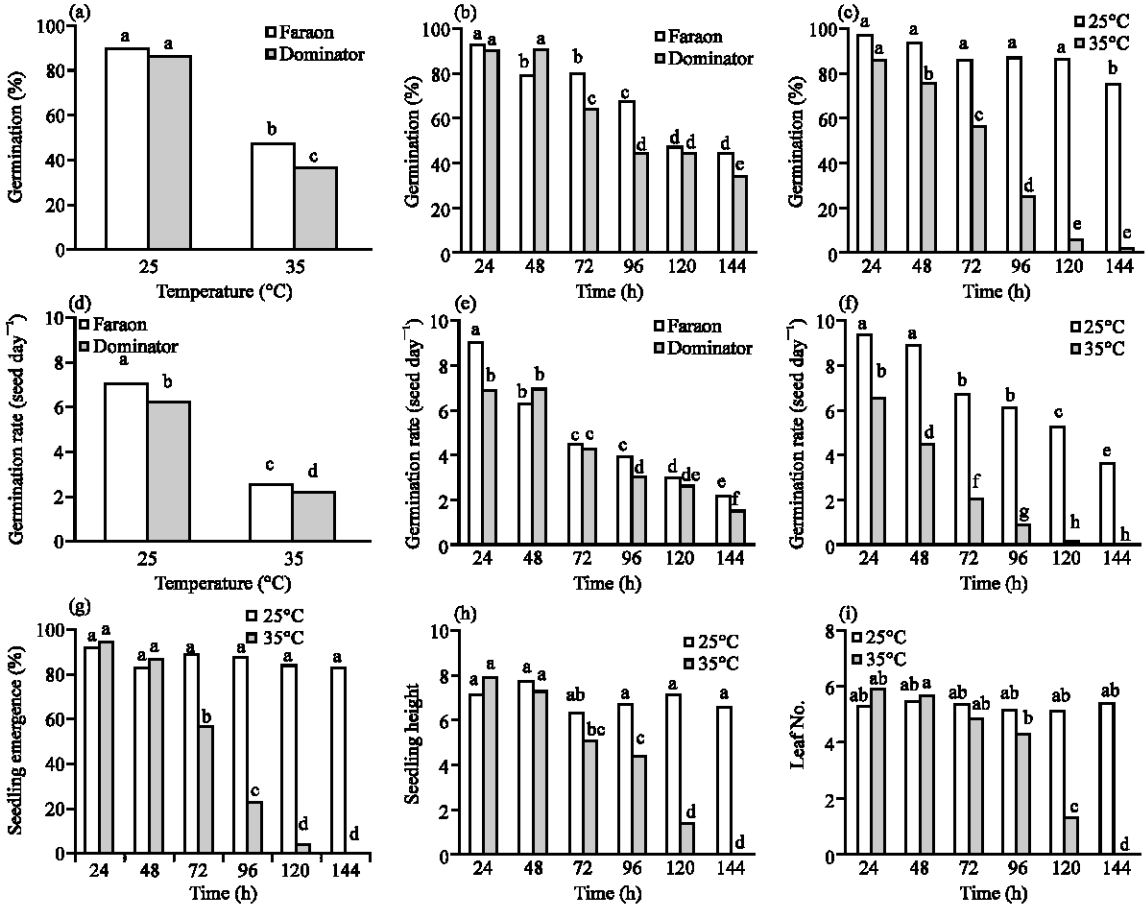

Fig. 1: (a-i) Change in percentage germination, germination rate, seedling emergence, seedling height and leaf No./seedling during different temperature and periods of fermentation (cvs. Faraon, Dominator)

showed lower germination than Faraon cultivar with only exception of 48 h in which there was no difference among two cultivars statistically for germination rate.

In agreement with this conclusion, Singh *et al.* (1985) also found that seed germination affected by cultivar and decreased with duration of fermentation, the best fermentation period was 24 and 48 h for the cultivars Punjab Chhuhara and Punjab Kesari, respectively.

Determination of interaction effects between fermentation time and temperature revealed that at 25°C, increasing the time up to 120 and 48 h had not significantly effect for percentage germination and germination rate, respectively. However at 35°C, percentage germination was decreased significantly with fermentation times longer than 24 h. In this temperature germination rate showed very similar pattern of response as percentage germination, but in general in lower values. None of the tomato seeds incubated at 35°C had high germination rate (Fig. 1c, f). The results are in agreement with those obtained by Liptay (1989) where the temperature for tomato fermentation used was ≥25°C, seed vigour declined severely, but at 15°C, more than 50% of the seeds were viable even after 25 days of fermentation. He found that optimum seed quality were obtained at 15- 30°C from 24-48 h fermentation, our results are tallying with his finding. Since rate of fermentation is highly temperature dependent (Agrawal, 1995), it is obvious that various fermentation temperature and duration can cause differences in seed germination. So, fermentation needs careful regulation to ensure optimum seed quality. Silva *et al.* (1982) reported that fermentation with longer term and higher temperature can greater reduce germination. In present study seed germination was declined at 35°C and this reduction that was increasingly severe with increasing fermentation period, can attributed to addition of fungi, discolouration, sprouting and heavy seed leachate (Jadli and Singh, 2009).

The results show that seedling emergence was similar in all times at 25°C, but it was greatly reduced with

fermentation longer than 48 h, at 35°C and completely inhibited by 144 h fermentation (Fig. 1g). This could be explained by the fact that high fermentation temperature causes injury to the seeds effecting seedling emergence (McDonald and Copeland, 1997; Jadli and Singh, 2009).

Similar to seedling emergence, seedling preliminary growth remained unchanged with further time at 25°C. However fermentation longer than 48 and 96 h at 35°C had a significant effect on seedling height as well as the number of leaf per seedling, respectively (Fig. 1h, i) and they declined severely with long-term fermentation.

According to the present results the temperature and time at which the tomato seed pulp fermentation is done is crucial for the retention of good seed quality. In both cultivars, seed germination decreased with increasing temperature from 25 to 35°C and increasing period from 24 to 144 h. Totally fermentation duration from 24 to 48 h at temperature 25°C, is recommended. High temperature and time ie 35°C is especially deleterious to the retention of a high level of seed quality, therefore in warm environment the fermentation must be for short time.

The effect of different extraction methods on seed quality: Similar to previous experiment, cultivar Faraon had significantly higher mean than cultivar Dominator in most seed extraction procedures of tomato (Table 2).

The results obtained with the HCL treatment indicate among the times of treatment, germination rate was significantly the highest for 20 min. Interaction effect between pH and time was found significant in hydrochloric acid but not in sulphuric acid ($p<0.05$) respecting both percentage germination and germination rate values. The highest percentage germination and germination rate measured in pH = 3 for 20 min (92.8% and 6.5 seed/day, respectively) in hydrochloric acid treatment (Fig. 2a, b). In pH = 1 the lowest germination was observed on seed treated for 10 min that shows in low pH, the treatment time should be longer in compare high pH.

In Sodium carbonate (10%) extraction method, germination rate decreased significantly with increasing duration of treatment from 24 to 48 h (Fig. 2c).

The effect of different extraction methods on percentage germination and germination rate of Faraon cultivar was significant. Maximum percentage germination (92.8%) was recorded for seeds extracted by hydrochloric acid extraction method (pH = 3, 20 min). However, the percentage germination was not significantly different in the most of seed extraction methods (Fig. 3a). Totally none of the extraction methods was detrimental to percentage germination of seeds and it was greater than 80% for all treatments, but the seeds extracted by the means of acid were shiny and cleaned quite easily in

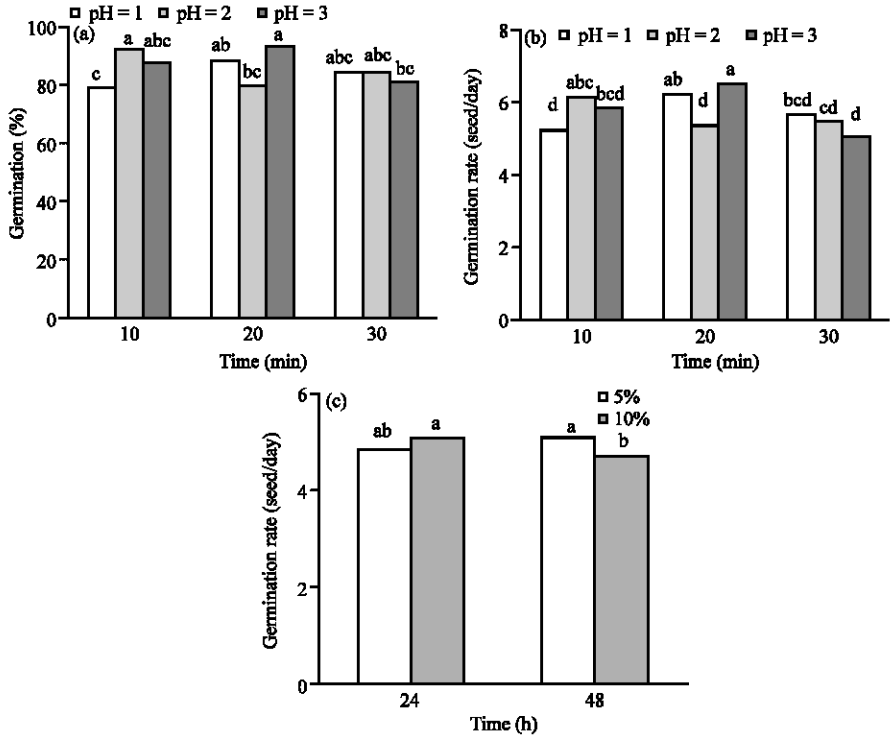

Fig. 2: Change in percentage germination and germination rate during different pH and time in HCL treatment (a, b), Change in germination rate during different concentration and time in Sodium carbonate treatment (c)

Table 2: The effect of seed extraction method on seed germination of two cultivar's tomato

Treatment	Germination (%)	Germination rate	Length of radicle	Length of plumule
HCl				
Cultivar				
Faraon	84.4a	5.8a	7.9a	4.5a
Dominator	86.5a	5.6b	7.7a	4.6a
pH				
1	84.1a	5.7a	7.6a	4.6a
2	85.4a	5.6a	8.0a	4.5a
3	87.0a	5.8a	7.9a	4.5a
Time (min)				
10	86.1a	5.7ab	7.9a	4.5a
20	87.2a	6.05a	7.5a	4.7a
30	83.1a	5.4b	8.0a	4.5a
H_2SO_4				
Cultivar				
Faraon	87.8a	6.1a	8.9a	4.8a
Dominator	91.1a	6.2a	7.3b	4.4b
pH				
1	87.2a	6.1a	8.5a	4.6a
2	89.7a	6.2a	7.7a	4.7a
Time (min)				
15	89.2a	6.1a	8.4a	4.6a
30	87.8a	6.1a	7.8a	4.6a
Na_2CO_3				
Cultivar				
Faraon	91.9a	5.6a	8.4a	4.5a
Dominator	85.8b	4.2b	7.7b	4.4a
Concentration (%)				
5	89.7a	5.0a	8.1a	4.5a
10	88.1a	4.9a	8.2a	4.6a
Time (h)				
24	89.2a	5.0a	8.1a	4.8a
48	88.6a	4.9a	8.2a	4.3a

Values with different letters are significantly different

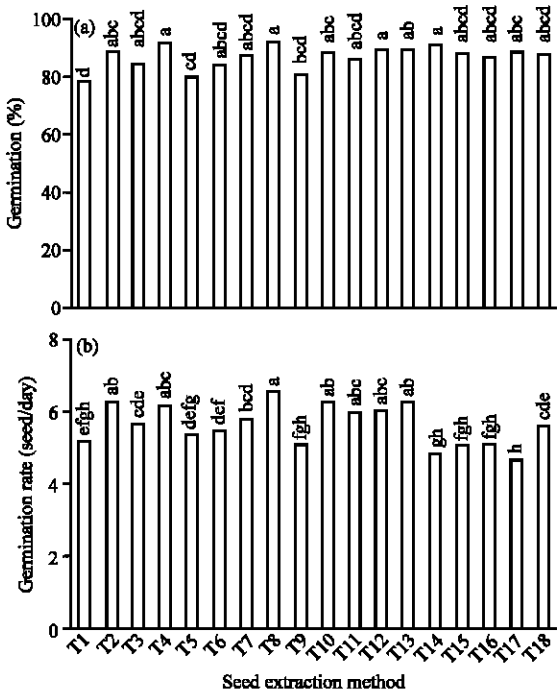

Fig. 3: The effect of different extraction methods on percentage germination and germination rate of Faraon cultivar (T1: HCl, pH = 1, 10'; T2: HCl, pH = 1, 20'; T3: HCl, pH = 1, 30'; T4: HCl, pH = 2, 10'; T5: HCl, pH = 2, 20'; T6: HCl, pH = 2, 30'; T7: HCl, pH = 3, 10'; T8: HCl, pH = 3, 20'; T9: HCl, pH = 3, 30'; T10: H_2SO_4, pH = 1, 15'; T11: H_2SO_4, pH = 1, 30'; T12: H_2SO_4, pH = 2, 15'; T13: H_2SO_4, pH = 2, 30'; T14: Na_2CO_3, 5%, 24 h; T15: Na_2CO_3, 5%, 48 h; T16: Na_2CO_3, 10%, 24 h; T17: Na_2CO_3, 10%, 48 h; T18: Natural fermentation)

compare to other methods since very good breakdown of coating occurred, while all alkali treatments showed dark testa that may reduce the preference by farmers. This agrees with conclusion of Brar and Singh (1984) and Yadav *et al.* (2004). Whereas Silva *et al.* (1982), Vadivelu and Srimathi (1988) and Jadli and singh (2009) reported that the seed extracted by various procedures respond differently to germination tests. They stated that natural fermentation was not satisfactory method in breaking down the seed gelatinous coating from the seeds and fermentation gave lower germination than acid treatments in their study. By contrast, Das *et al.* (1997) and Olympio and Dankyir (1999) reported that the best quality seed was observed for seeds extracted using the fermentation technique. On the other hand acid treatment in Vadivelu and Srimathi (1988) and Gowda *et al.* (1991) studies, alkali treatment in Ghosh and Syamal (1997) study and HCL and alkali treatments in Jadli and Singh (2009) experiment considered as the best extraction method. These differences may be attributed to the difference in initial seed quality and or may be due to difference in seed coat permeability, thickness and leaching out of different amino acids (Jadli and Singh, 2009). The seed quality parameters although differed significantly due to seasons, environmental and varietal differences, effect of chemicals type and concentration used for seed extraction, application period of treatments, various seed quality and vigour tests.

The variation in speed of germination under different treatments was also observed (Fig. 3b). Results showed that germination rate higher in those treatments where fruit pulp was treated with acid. Soaking in HCl (pH = 3) for 20 min resulted in the highest germination rate (6.5 seed/day). However, reduced germination rate was recorded with alkali and fermentation treatments. These finding are in close conformity with the observations of Silva *et al.* (1982) and Jadli and Singh (2009) which obtained the highest germination rate with HCl extraction method.

Length of radicle and length of plumule has been studied during the investigation and no significant variations among the treatments were observed.

On the basis of above results it can be conducted that HCl treatment had created ideal conditions for germination of tomato seeds.

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