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Evaluation and Selection of Open-pollinated Tomato (*Solanum lycopersicum* L.) Entries for Adaptation under Temperate Agroecological Conditions of Bhutan

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ABSTRACT

Lack of high-yielding climate-resilient varieties and, frequent pest and disease incidences are the major issues in tomato production in Bhutan. The National Centre of Organic Agriculture, Yusipang, introduced 40 open-pollinated entries between 2020 and 2021. With an objective to evaluate and select the most desired tomato varieties for commercial cultivation in Bhutan, seven tomato entries were prescreened and selected using combined scoring of the total votes from the Participatory Varietal Selection and yield of entries in 2021. The Randomized Block Design with eight entry treatments and three replications with Ratan as standard check was employed to evaluate their yield and yield parameters; fruit quality; and tolerance to pests and diseases in 2022. ANOVA followed by Tukey HSD test for mean separation was employed at p-value at P<0.05. The result showed that AVTO1954 produced a significantly higher yield (29.8 tons/acre) compared to AVTO1910 but not significantly different from AVTO1702 (28.5 tons/acre) and AVTO1907 (28.4tons /acre). Although Roma (check) produced the highest total number of fruits per plant (110), it produced the lowest number of marketable fruits per plant (7) compared to all other entries, while the plant height did not show any statistically significant differences between different treatment entries. Two entries with the lowest disease incidence were AVTO1702 and AVTO1954, while Roma was infested with blight and powdery mildew at 27% and 45% respectively. The study recommends the release of three entries viz-a-viz AVTO1954, AVTO1907 and AVTO1702 and similar research in other agroecological zones of Bhutan to identify appropriate varieties in their zones.

Keywords: *Tomato; Germplasms; Selection; Participatory Varietal Selection*

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1 Introduction

Tomato (*Solanum Lycopersicum* L.) is one of the most widely cultivated and consumed vegetable crops globally (Bihon et al., 2022). It is generally consumed fresh, cooked, or processed into various products such as ketchup and paste (Rawal et al., 2017). It is rich in minerals, ascorbic acids, organic sugars and fibres including vitamins B and C (Ali et al., 2016). The lycopene in tomatoes is reported to be one of the most potent antioxidants, which plays an important role in the prevention of certain forms of cancer and cardiovascular disease (Agarwal & Rao, 2000).

Tomato is one of the priority crops identified by the Department of Agriculture, Ministry of Agriculture and Forests (DoA, 2017). Although production has gradually increased over the years with about a 10 percent jump from 2016 to 2021, domestic supply is still too low to meet the country's demand. The total cultivated area covered by tomatoes was 229 acres with a total production of 289 tons in the year 2021 (NSB, 2022). Consequently, in the same year, Bhutan imported 4296.63 tons with a corresponding value of about Nu. 150 million to fill in the supply gap making it one of the highest imported vegetables in the country (MoF, 2021).

Bhutan has a high potential to enhance tomato production to meet domestic demand owing to its diverse agroecological conditions. In Bhutan, tomatoes are cultivated under protected structures to enhance production by protecting the crops from pests and diseases; compensate for low temperatures in high altitudes (Sotelo-Cardona, Lin, & Srinivasan, 2021). However, tomato production in Bhutan is constrained by a lack of high-yielding varieties, frequent pest and disease outbreaks and unorganized marketing channels. The major disease affecting tomato cultivation in Bhutan is a fungal disease called blight (NPPC, 2017). The production season of tomatoes coincides with the rainy season which makes it conducive for fungus to thrive and affect the tomato plants. The problem is more severe in organically managed farms due to no or limited organic inputs available to control the blight disease (Singh et al., 2021). Further, Bhutan has only two officially released varieties namely Ratan and Roma which are degenerated and become highly susceptible to blight with shorter fruit shelf life and low yield as it was released in 2002 (DoA, 2017). As an alternative effort to increase tomato production in the country, hybrid tomatoes namely *Cosmic* and *Garv* were tested for adaptability and performance and are being promoted. However, like elsewhere, the sustainability, as well as the cost of such imported hybrids, remains a serious concern (Kutka, 2011).

It has therefore become necessary for Bhutan to initiate proper research to select sustainable and economical tomato varieties with desired traits for promotion in the farmer's field. One such method is through the introduction and evaluation of open-pollinated varieties which are sustainable due to their ability to produce seeds year after year with improved adaptation (Gotame, Gautam, Ghimire, & Shrestha, 2021). They are also considered to be an important source of plant materials for future breeding programs. Furthermore, open-pollinated varieties are preferred for organic farming due to their high adaptation and climate-resilient capabilities. The main objective of the current study was to evaluate and select the best varieties with desirable traits of high yields, disease resistance and adaptability to Bhutan's climatic conditions.

2 Materials and Method

2.1 Study location and details

The study was conducted at the National Center for Organic Agriculture, Yusipang (NCOA) which is in the cool temperate agroecological zone (latitude of 27 º27'50" N and longitude of 89 º42 '25 "E) at an elevation of 2700 meters above sea level. It receives an annual rainfall ranging between 50 mm to 650mm with an annual mean temperature of about 12.5 °C. The study was conducted over a period of three years from March 2020 to September 2022. In addition, an evaluation was conducted at two locations (Ramthangkha and Bjemina villages of Paro and Thimphu Dzongkhag respectively) to understand the performance of selected varieties in the farmer's field.

2.2 Tomato entries

A total of 40 tomato entries were introduced for evaluation and selection during the study duration from the World Vegetable Centre based in Taiwan. In the first year (2020), 23 entries were evaluated under open field. However, almost all the entries except six entries from the open field cultivation were lost to blight disease due to which the studies in the following years were conducted under the greenhouse condition. In the second year, six entries rescued from the first-year cultivation trials along with 17 new entries were cultivated on a standard bed size of 2 m x 1 m without any replication inside greenhouses.

2.3 Pre-screening and ranking of entries

The data from the Participatory Varietal Selection (PVS) method and the yield from the experiment were used to perform the pre-screening of the most desired entries. The PVS was done involving farmers from Yusipang and Hongtsho communities who have some experience in tomato farming and marketing. The ranking of entries was done by ranking the sum of

standardized scores calculated by addition of standardized values of total votes and yield (Table 2). The rows values from PVS and yield (Table 1) were normalized using the equation (Eq. i). The top seven entries were selected for further evaluation following replicated trial in the final year (2022).

$$X_{Scaled} = \frac{X - X_{min}}{X_{max} - X_{min}} \qquad \text{Eq. i}$$

Whereas,

X_{Scaled} is standardized values

X is the row values of total votes/yield

X_{min} is the minimum values of array of row values

X_{max} is the maximum value of array of row values

Table 1. Raw vote and yield data from PVS and field trial

Entry number	Growth habit	Vote Count (Male)	Vote Count (Female)	Total Votes	Yield (Tons/Acre)
AVTO1954	D	96	52	148	10.06
AVTO1907	D	50	10	60	16.72
AVTO1003	D	57	15	72	14.90
AVTO1705	D	38	45	83	13.00
AVTO1911	D	42	27	69	13.29
AVTO1702	D	75	48	123	5.87
AVTO1910	D	61	39	100	7.20
AVTO1909	D	39	25	64	9.95
AVTO1919	D	10	9	19	13.22
AVTO1008	D	11	5	16	10.79
AVTO1912	SD	8	7	15	10.61
AVTO1921	SD	10	12	22	7.95
AVTO1828	ID	0	0	0	10.06
AVTO1915	SD	10	8	18	7.64
AVTO1903	D	13	7	20	7.18
AVTO1010	D	15	4	19	6.20
AVTO1315	NA	0	0	0	7.33
AVTO1913	SD	8	6	14	4.98
AVTO1706	SD	0	0	0	4.09
AVTO1914	SD	0	0	0	3.78
AVTO1288	NA	0	0	0	2.69

AVTO0301	SD	0	0	0	1.58
AVTO1829	ID	0	0	0	0.00

D = Determinate; SD = Semi determinate; ID = Indeterminate; NA = Not Available

Table 2. Rank, normalized scores for total votes, yield and sum of normalized values of tomato entries

Entry number	Normalized votes	Normalized yield	Sum of score	Rank
AVTO1954	1.00	0.60	1.60	1
AVTO1907	0.41	1.00	1.41	2
AVTO1003	0.49	0.89	1.38	3
AVTO1705	0.56	0.78	1.34	4
AVTO1911	0.47	0.79	1.26	5
AVTO1702	0.83	0.35	1.18	6
AVTO1910	0.68	0.43	1.11	7
AVTO1909	0.43	0.60	1.03	8
AVTO1919	0.13	0.79	0.92	9
AVTO1008	0.11	0.65	0.75	10
AVTO1912	0.10	0.63	0.74	11
AVTO1921	0.15	0.48	0.62	12
AVTO1828	0.00	0.60	0.60	13
AVTO1915	0.12	0.46	0.58	14
AVTO1903	0.14	0.43	0.56	15
AVTO1010	0.13	0.37	0.50	16
AVTO1315	0.00	0.44	0.44	17
AVTO1913	0.09	0.30	0.39	18
AVTO1706	0.00	0.24	0.24	19
AVTO1914	0.00	0.23	0.23	20
AVTO1288	0.00	0.16	0.16	21
AVTO0301	0.00	0.09	0.09	22
AVTO1829	0.00	0.00	0.00	23

2.4 Nursery management

Seeds were sown in the March under polytunnels to protect the juvenile seedlings from insect infestation and other external factors such as wind, rain, etc. Nursery beds of dimension 2 m x 1 m were prepared by ploughing the land using a power tiller followed by mixing the fine soil with organic manure. Brief irrigation was done after sowing to facilitate the germination of the seeds. Seedlings were hardened for a week when the seedling attained about 8 cm to 10 cm in height with at least five number of leaves before transferring to the greenhouses.

2.5 Study design

The study was conducted under greenhouses of 20 m x 5 m dimensions. The trial was laid out in a Randomized Complete Block Design (RCBD) with 8 treatments of tomato entries which were replicated 3 times. The 8 treatment plots of raised beds with the dimensions of 2.5 m x 1.2 m were prepared with a spacing of 0.30 m in between to facilitate intercultural operations. All the treatment plots were then mulched with black mulching plastics followed by drilling 24 numbers of holes in each plot at a plant-to-plant distance and row-to-row distance of 0.40 m and 0.60 m respectively between rows. Tomato varieties namely Roma which is an open-pollinated and released variety was used as a standard check.

2.6 On-farm trial

The on-farm trials were conducted in two districts namely Paro and Thimphu to understand the performance of selected entries under farmer's management conditions. The treatment plots were prepared with the same dimensions, mulching materials, and plant-to-plant and row-to-row distance as that followed in the on-station trial. However, it was not possible to establish the desired replicated trials in the farmer's field due to limited polyhouses and farmers' preferences for growing other crops such as chili during the season.

2.7 Data Collection

A standard data collection format was adopted from Hanson et al. (2011). Data on yield and yield parameters, disease resistance, and their fruit characteristics were collected from five randomly selected plants from each treatment plot. Yield and yield parameters included the number of total fruits, number of marketable fruits, number of non-marketable fruits, the weight of marketable fruits (g), and weight of non-marketable fruits (g). Plant height was measured using a measuring tape from five sample plants from each treatment plot during the last harvest. The growth habit (determinate, indeterminate, semi-determinate) of the tomato entries was recorded consistently during the study period. The incidences of disease and pests such as late blight, powdery mildew, yellow leaf curl disease, bacterial wilt, fruit borer and collar rot were recorded on fortnightly basis. The disease and pest incidence in the treatment plot was calculated using the formula in the equation (Eq. ii).

$$PDI\ (\%) = \frac{NIPs}{TNP}\ x\ 100 \qquad \text{Eq. ii}$$

Whereas,

PDI (%) is the pest and disease incidence in a treatment plot in percentage

NIPs is the total number of infested or infected plants in a treatment plot

TNP is the total number of plants in a treatment plot

Fruit characteristics such as fruit weight (g), fruit diameter (mm) and fruit length (mm) were measured from 10 randomly selected fruits at each harvest. While segregating marketable and non-marketable fruits, the following conditions were considered.

a) Fruits free from blemish and defects which weighed more than 30 grams with an equatorial diameter of more than 35 mm were categorized as "marketable fruits".
b) Fruits which weighed less than 30 grams with a diameter of less than 35 mm and the ones with defects or blemishes were categorized as "non-marketable fruits".

2.8 Data analysis

Raw data collected were entered, cleaned, and preprocessed in Microsoft Excel 365. Data were statistically analyzed in R statistical software version 4.2.2 (R Core Team, 2022) using a one-way analysis of variance (ANOVA) followed by mean separation using Tukey Honest Significance Difference test. The alpha value was set at $P = 0.05$ to detect statistically significant differences between all the comparisons made in this article. The R-packages such as 'tidyverse' version 1.3.2 (Wickham et al., 2019), 'ggstatsplot' version 0.9.5 (Patil, 2021), 'dlookr' version 0.6.0 (Ryu, 2022), and 'car' version 3.1.1 (Fox & Weisberg, 2019) were used for data wrangling, cleaning and analysis. The graphical representations of the statistical data were prepared using r-package 'ggplot2' version 3.3.6 (Wickham, 2016).

3 Results and Discussion

3.1 Yield of tomato entries

It is evident from the result (Figure 1) that AVTO1954 produced a significantly higher yield (29.8 tons/acre) compared to AVTO1910 (9.91 tons/acre) while it did not show any statistically significant difference with other tomato entries including the check variety. The yields were not significantly different between entries AVTO1702 (28.50 tons/acre), AVTO1907 (28.4 tons/acre), AVTO1705 (27.50 tons/acre), AVTO1003 (22.10 tons/acre), AVTO1911 (19.20 tons/acre) and Roma (20.30 tons/acre). On the other hand, yields of entries AVTO1003, AVTO1911, Roma and AVTO1910 were statistically similar. Our results also match with a report from the World Vegetable Center (World Veg, 2022) on improved tomato lines according to which the AVTO1954 is considered blight resistance and most suitable for cool and wet conditions. The low total yield in AVTO1910 is due to weak plants and poor adaptability leading to high mortality after transplanting and throughout the growing season.

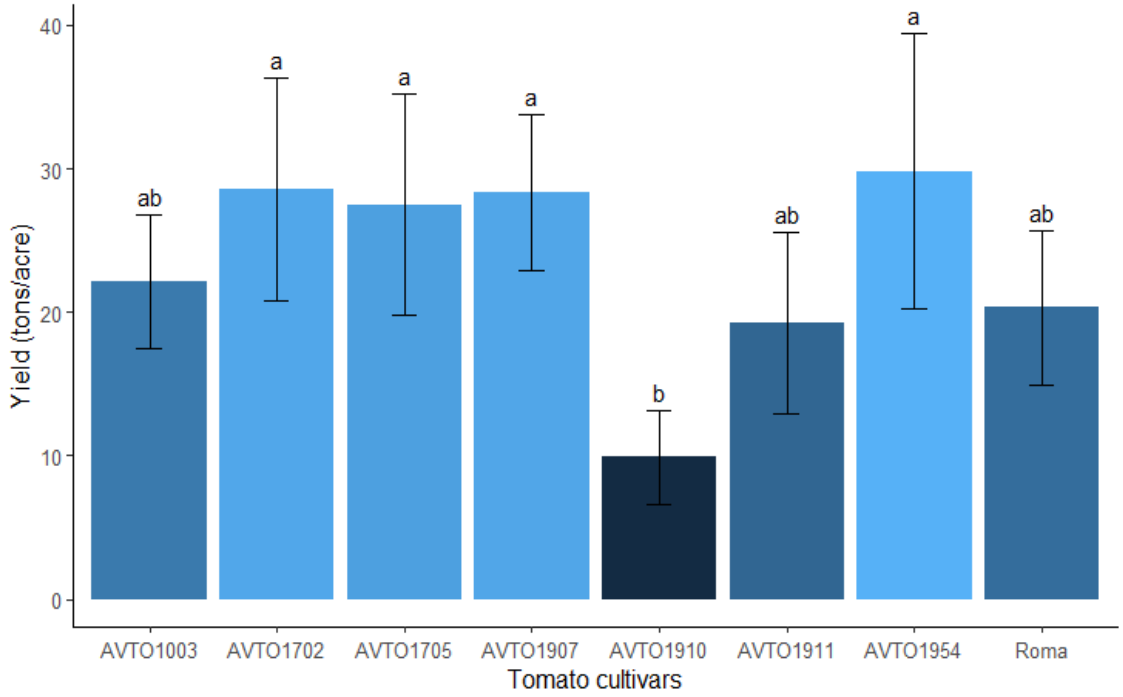

Figure 1. Yield (Tons/Acre) of tomato entries. Different lower-case letters indicate statistically significant differences following Tukey's HSD post hoc test at $P<0.05$

3.2 Total number of fruits per plant

The result (Figure 2) revealed that the total number of fruits per plant was highest in Roma (110) which was significantly higher than AVTO1911 (51) and AVTO1910 (31) but not significantly different from AVTO1907 (89), AVTO1954 (89), AVTO1702 (87), AVTO1705 (84) and AVTO1003 (59). The total number of fruits produced by AVTO1911 was not significantly different from that of AVTO1910, AVTO1003 and AVTO1705. The result also clearly indicated that the number of fruits produced by AVTO1907, AVTO1954, AVTO1702, AVTO1705, AVTO1003 and AVTO1911 was not significantly different from each other. The result shows that Roma may be a very good fruit bearer, but it gives poor fruit quality which may not be profitable to our farmers (Figure 43, 4). The result also indicated that the variety Roma is the least productive under protected conditions.

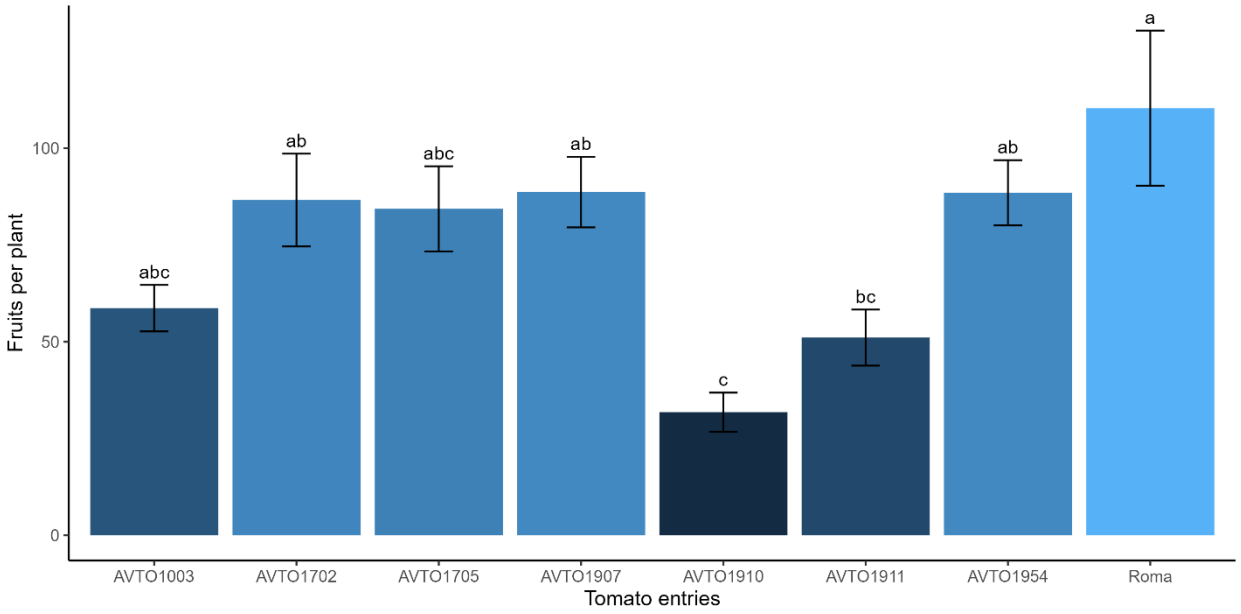

Figure 2. Total fruits/plant of different tomato entries. Different lower-case letters indicate statistically significant differences following Tukey's HSD post hoc test at $P<0.05$

3.3 Number of marketable fruits per plant

As shown in (Figure 3), there were highly significant differences in terms of the number of marketable fruits of AVTO1954 (39) and AVTO1907 (35) compared to the check variety Roma (7) at $P<0.05$, however, they were not significantly different to that of AVTO1705 (33), AVTO1702 (29), AVTO1911 (28), AVTO1003 (27), and AVTO1910 (18). Further, the total number of marketable fruits per plant from AVTO1705, AVTO1702, AVTO1911, AVTO1003, and AVTO1910 was not significantly different from each other. The result confirms that the check variety Roma is degenerated and no more suitable for commercial cultivation.

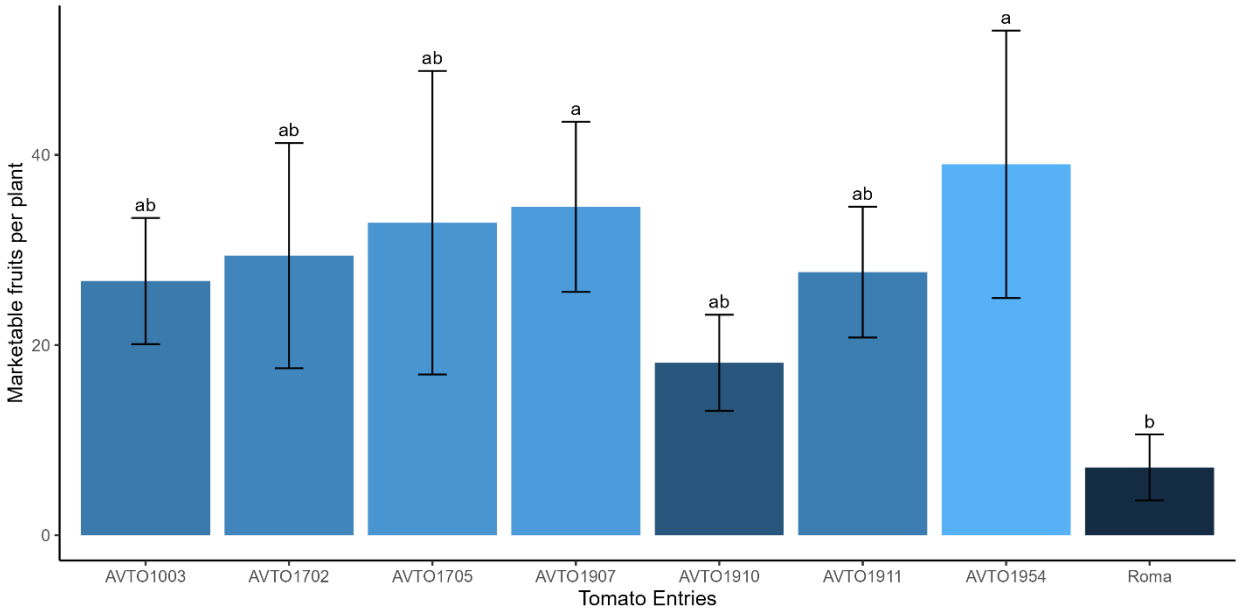

Figure 3. Number of marketable fruits/ per plant. Different lower-case letters indicate statistically significant differences following Tukey's HSD post hoc test at $P<0.05$

3.4 Number of non-marketable fruits per plant

The result (Figure 4) of the total number of non-marketable fruits per plant produced by different tomato entries shows that the check variety Roma (103) produced a highly significant number of non-marketable fruits compared to all other tomato entries except AVTO1702. In contrast, the total number of non-marketable fruits per plant from AVTO1702 (57), AVTO1907 (54), AVTO1705 (51), AVTO1954 (49), AVTO1003 (32), AVTO1911 (23) and AVTO1910 (13) were not statistically different. The result consistently confirmed that the performance of new entries of tomatoes is better compared to the check variety.

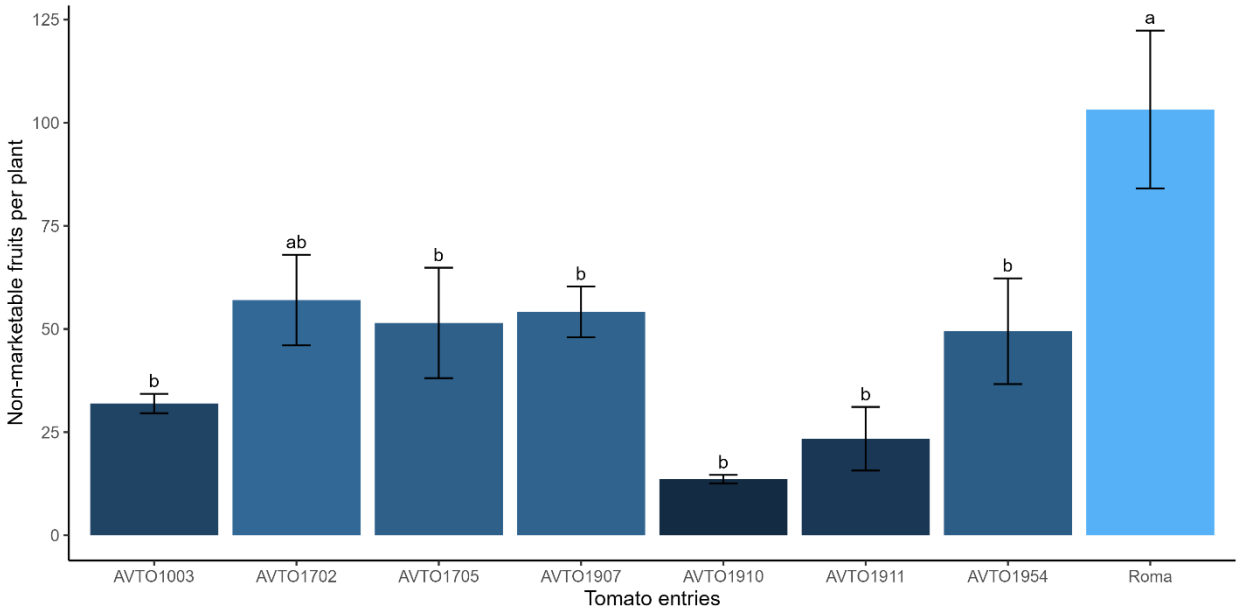

Figure 4. Total number of non-marketable fruits per plant of tomato entries. Different lower-case letters indicate statistically significant differences following Tukey's HSD post hoc test at $P<0.05$

3.5 Fruit characteristics of tomato entries

The mean values of fruit length (mm), fruit diameter (cm), fruit weight (g) and corresponding fruit shapes are presented in (Table 3). It revealed that the tomato entry AVTO1907 produced a significantly longer fruit length compared to all other entries with the shortest fruit length from Roma. Further, the fruit length of all the entries were found to be significantly different from each other except between AVTO1702 and AVTO1910; AVTO1910 and AVTO1705.

The AVTO1954 and Roma obtained fruits with the widest and narrowest diameter respectively. The fruit diameter of AVTO1954 was significantly different from AVTO1003, AVTO1907 and AVTO1910, and not from AVTO1702, AVTO1705 and AVTO1911 while the Roma obtained fruits with significantly narrow widths compared to all other entries. However, tomato entries (AVTO1702, AVTO1705, AVTO1907, AVTO1910, and AVTO1911) obtained statistically similar fruit width.

The fruits from AVTO1911 were significantly heavier compared to that from AVTO1003, AVTO1705, AVTO1954 and Roma, however, they were of statistically similar weight with fruits from AVTO1702, AVTO1907 and AVTO1910. The fruit of AVTO1954 was significantly heavier than that of AVTO1705 and Roma. On the contrary, Roma produced significantly lightest fruits compared to all other entries under study.

Table 3. Mean (SE) of fruit length, fruit width, fruit weight and fruit shape of different tomato entries

Treatment	Mean fruit length (mm)	Mean fruit width (mm)	Mean fruit weight (g)	Fruit shape
AVTO1003	57.09(1.02)^{b}	39.14(0.09)^{c}	52.63(2.2)^{b}	Oblong
AVTO1702	53.1(0.63)^{d}	45.16(0.75)^{ab}	64.93(0.55)^{ab}	Oblong-blocky
AVTO1705	51.23(0.39)^{e}	45.3(2.87)^{ab}	52.33(2)^{c}	Round, pointed tip
AVTO1907	58.88(0.22) ^{a}	42.89(0.13)^{b}	60.03(1.17)^{ab}	Oblong
AVTO1910	51.79(1.15)^{de}	42.77(0.44)^{b}	58.7(4.37)^{ab}	Oblong
AVTO1911	55.08(0.11)^{c}	44.55(0.51)^{ab}	68.77(0.45)^{a}	Blocky to oblong
AVTO1954	42.45(0.06)^{f}	46.39(0.38)^{a}	53(2.96)^{b}	Blocky
Roma (check)	30.15(0.13)^{g}	34.1(1)^{d}	29.87(5.23)^{d}	Oblong
F-Statistics	*712*	*38.27*	*38.27*	
P-value	*<0.001*	*<0.001*	*<0.001*	

Different lower-case letters in the superscript indicate statistically significant differences following Tukey's HSD post hoc test at $P<0.05$

3.6 Plant height of tomato entries

The mean plant height of different tomato entries measured during the last harvest is subjected to the Analysis of Variance test to see if there is a statistical difference in the height of different entries tested in this study (*result not shown*). Although there is no significant difference in the plant height of all the entries, the tallest plants were obtained from AVTO1911 (103.5 cm) followed by AVTO1003 (102.4 cm) and AVTO1705 (102 cm), AVTO1954 (95.20 cm), AVTO1702 (94.50 cm), AVTO1907 (93.10 cm), Roma (92.90 cm). The shortest plants were obtained from AVTO1910 with a mean plant height of 73.10 cm.

3.7 Pests and disease incidence

As the experiment was conducted under greenhouse conditions, the incidences of pests and diseases were very low to cause any significant reduction in crop growth and yield. The minor incidences of powdery mildew and blight diseases observed were recorded and the result is presented in table (Table 4). The result shows that the check variety Roma had a comparatively higher level of disease infection with the severity score of powdery milder and blight of 45% and 27% respectively, while other entries some incidences of diseases with no sign of blight incidence in AVTO1702 and AVTO1954.

Table 4. Pest and disease incidence (Blight and powdery mildew)

Treatments	Blight				Powdery mildew			
	TNP	NHP	NIP	PDI (%)	TNP	THP	TIP	PDI (%)
AVTO1003	24	20	2	10	24	20	4	20
AVTO1705	24	21	3	14	24	21	4	19
AVTO1907	24	24	2	8	24	24	6	25
AVTO1702	22	20	0	0	22	20	6	30
AVTO1911	19	19	3	16	19	19	6	32
AVTO1954	23	20	0	0	23	20	8	30
AVTO1910	17	16	3	18	17	16	4	25
Roma	20	18	5	27	20	18	9	45

3.8 Yields (tons/acre) of tomato entries from the on-farm and on-station trial

The mean yield (tons/acre) of introduced and selected tomato entries from all three study sites are presented in figure (Figure 5). Overall, all the tomato entries yielded the lowest at Bjimina while yields obtained from Ramthangkha and Yusipang were similar for all the entries except AVTO1910. The result from Ramthangkha showed that AVTO1954 (39 tons/acre) yielded the highest followed by AVTO1702 (29 tons/acre), AVTO1907 (28.90 tons/acre), AVTO1003 (27.10 tons/acre), AVTO1705 (25.50 tons/acre), AVTO1911 (23 tons/acre), while AVTO1910 obtained the lowest yield of 20 tons/acre. Similarly, at Bjimina, AVTO1705, AVTO1702 and AVTO1954 obtained the highest mean yield of 11 tons/acre followed by AVTO1910 (10 tons/acre), AVTO1003 and AVTO1907 with the same yield of 9 tons/acre, while AVTO1911 produced the lowest yield of 6 tons/acre. Furthermore, results from Yusipang showed that AVTO1954 gave the highest yield of 29.8 tons/acre followed by AVTO1702 (28.5 tons/acre), AVTO1907 (28.4 tons/acre), AVTO1705 (27.5 tons/acre), AVTO1003 (22 tons/acre), AVTO1911 (19.2 tons/acre) and lowest yield from AVTO1910 with 10 tons/acre. The consistently low yield in tomato entries at Bjimina was due to taking record of only the first three harvests by the farmers after which the crops were uprooted for leguminous vegetables and Cole crops. The findings confirm that the three entries (AVTO1954, AVTO 1705 and AVTO1907) have consistently shown higher yields in all three locations which can be identified for further cultivation on a mass scale by tomato farmers.

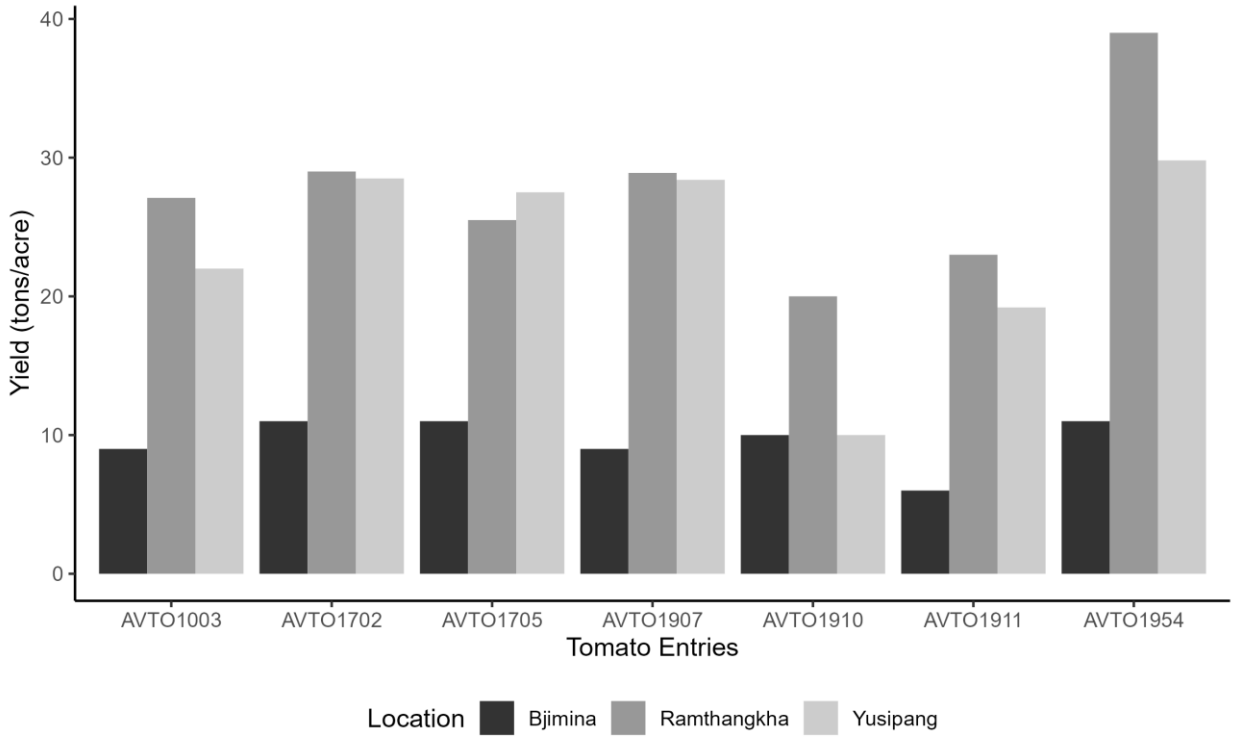

Figure 5. Mean yield (tons/acre) of AVRDC tomato entries at three study sites

4 Conclusion

The present investigation and research revealed that the tomato varieties introduced by the World Vegetable Centre, Taiwan are superior in terms of marketable yield, fruit quality and disease resistance compared to the standard check (Roma). The overall yield of AVTO1954, AVTO1702, AVTO1907 and AVTO1705 were significantly higher compared to AVTO1910. However, all the new entries produced a significantly higher number of marketable fruits compared to the check variety. Although the total number of fruits of standard check (Roma) was significantly higher compared to AVTO1910 and AVTO1911, no statistical difference was observed with other entries. However, Roma obtained the lowest significant number of marketable fruits compared to AVTO1954, and a significantly higher number of non-marketable fruits compared to all other entries. Therefore, we can conclude that for the commercial promotion of varieties, it is not the total yield that is important but the total number of marketable fruits which is more critical. In addition, tomato entries viz-a-viz AVTO1954, AVTO1702 and AVTO1907 could be selected for promotion and commercial production in temperate conditions of Bhutan. The result from the on-farm trials also confirms our conclusion.

We recommend that proper research focusing on pest and disease resistance be conducted with these entries to draw a scientifically concrete conclusion on their response to major tomato pests and diseases. Furthermore, similar research in the other agroecological zones in Bhutan is recommended to select appropriate entries for respective zones.

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