



Effect of shade intensities and chilling duration on quality fruit and runner production of strawberry

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Abstract

Strawberry cv. Chandler is the only commercial cultivar in Pakistan which is majorly propagated through crowns. Its production is highly effected by temperature that is why a field experiment entitled “effect of shade intensities and chilling duration on quality fruit and runner production of strawberry” was undertaken at Agriculture Research Institute Swat during 2017-2018. The experiment was triplicated using randomized complete block design with split plot arrangement. Shade intensities (0, 30%, 50%, 70%) were kept in main plot while pre transplant chilling duration (0, 3, 6 and 9 days) at $4\pm 1^{\circ}\text{C}$ were subjected to subplots. The results showed maximum value for number of crowns plant⁻¹ (32.6) and crown diameter (9.6mm) under 30% shade level while maximum plant height (31.1cm) was observed under 70-75% shade intensity. Yield and qualitative attributes like single fruit weight (12.6g), total yield (196.7g m⁻²), ascorbic acid (56.3mg 100g⁻¹), total soluble content (10.3 °brix), titratable acidity (1.0%), reducing sugar (5.0%) and non-reducing sugar (1.7%) were maximum under open field condition. Regarding chilling duration, higher values for plant height (28.7cm), single fruit weight (12.5g) and total yield (186.9g m⁻²) were observed in plants who received 6 days pre-transplant chilling duration while number of crowns plant⁻¹ (27.3) and crown diameter (9.8mm) was recorded in plants subjected to 9 days pre-transplant chilling duration. It is concluded that for increase in crowns (daughter plants) production, strawberry cultivation should be done under 30% shade intensity with strawberry plants subjected to 6-9 days pre transplant chilling, while for qualitative as well as quantitative increase in fruit yield, open field along with 6 days pre-transplant chilling duration is recommended.

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Introduction

Strawberry is a nutritious crop produced in almost all provinces of Pakistan (Dad, 2011). Like other horticultural crops, its successful production depends upon several factors including supply of high quality planting material and temperature. Using low quality plants leads to irreversible losses that the grower cannot earn back (Hatfield and Preuger, 2015; Massetani and Neri, 2016). The ideal temperature to produce high quality strawberry is between 20 and 26°C; temperatures below 20°C slow down the growth and development of both the strawberry plant and fruit, whereas temperatures above 35°C cause the strawberry plant to stop growing and may succumb to heat stress (Galletta and Bringhurst, 1990). As expected by Intergovernmental Panel Climate Change (IPCC, 2007), the expected changes in temperature over the next 30-50 years are predicted to be in the range of 2-3°C. Thus, heat stress is a challenge in strawberry production that has been reported all over the world (Chen, 2013).

Several practices can be used to cope with temperature stress and shade netting is one technique used to reduce solar radiation, temperature, and ensure the optimal microclimate for the crop (Ambrozy *et al.*, 2015). Plants optimize their growth and development in a given environment by responding to light quality, quantity, direction and periodicity (Oren-Shamir *et al.*, 2001). Physiological factors like photosynthesis, evapotranspiration, respiration, water absorption and mineral elements and their transport are more effectively utilized under shade thus increasing precocity and productivity and enabling off season production (Lobos *et al.*, 2013). Shading had significant effect on glucose and sucrose concentrations (Watson *et al.*, 2002). Previous researches suggested that light shade screens increased quality and productivity of various crops like of tomato, pear (*Pyrus sp.*), apple (*Malus domestica*), strawberry (*Fragaria ananassa*), grape (*Vitis vinifera*), and chili pepper (*Capsicum*) by increasing water and radiation use efficiency in summer seasons (Rajapakse and Shahak, 2007; Shahak *et al.*, 2008).

Another option to improve seedling quality is pre-transplant vernalization. Vernalization or pre-transplant chilling is the induction of a plant's reproductive process through natural or artificial exposure to chilling temperatures (e.g., 0 - 7°C). Chilling requirements vary from cultivar to cultivar (Bolda, 2008). Artificial vernalization of seedlings is a strategy to increase floral differentiation and increase fruit production (Costa *et al.*, 2014). Low temperatures improve the quality of seedlings due to a higher accumulation of reserves in the crown and roots of plants grown in this climate (Ruan *et al.*, 2009). Seedlings subjected to insufficient number of hours of cold have low yield (Wrege *et al.*, 2007). Runnering has also been shown to increase in day-neutral cultivars through vernalization (Robert and Rice, 1990).

Keeping in view the importance of shade and vernalization the present experiment was designed to accomplish the prominent role of pre-transplant chilling duration and shade intensities on growth, yield and quality traits of June-bearing strawberry cultivar Chandler under the agro climatic conditions of District Swat.

Materials and methods

The experiment was conducted at Agriculture Research Institute (ARI) Swat during the year 2017-2018. It is located at 34.7717°N latitude, 72.3602°E longitude with an attitude of 936 m above mean sea level. The soil of experimental site was Silt loam with a pH 6.6 (Soil Laboratory ARI Swat). Crowns of strawberry cv Chandler were collected from ARI Swat. The experimental designed in randomized complete block with split plot arrangement with four shade intensities and four chilling treatments replicated three times. Shade intensities (0, 30%, 50%, 70%) were kept in main plot while pre transplant chilling duration (0, 3, 6 and 9 days) at 4±1°C were subjected to subplots. Total of 48 plots were studied in the whole experiment having 16 treatments in each replication. Each treatment was having 8 daughter plants thus total 384 plants were used in the experiment.

The field was ploughed twice two weeks before the planting in both the years. The experimental area was prepared by cultivating it with rotavator a day before planting. Beds of 122 cm width and 31 cm height were formed manually with 61 cm bed to bed distance. Shade was applied 2 feet above the ground. Healthy runners of strawberry cv. Chandler collected from last year strawberry runners harvest at ARI Swat was used. Runners were transplanted having 6 inches plant to plant distance on both sides of beds with flood irrigation in furrows between the beds. Pre emergence herbicide Pend methylene was sprayed @7ml L⁻¹ during primary irrigation. Both organic and in-organic fertilizers were applied to the experimental field. Well-rotten Farm Yard Manure (FYM) was applied @ 3600kg acre⁻¹ just after the soil was ploughed a week before planting. After three to four days of transplantation irrigation was done again. After 30-45 days weeding was done and during weeding chemical fertilizer was applied @ 12:42:27 NPKkg ha⁻¹ in the form of Di ammonium phosphate (DAP). Corrective measures were taken for the control of pest and diseases. Replacement for any dead plant was done within 2 weeks after sprouting was replaced. Data was collected on plant height, number of crowns (daughter plants) plant⁻¹, crown diameter (mm), single fruit weight (g), total yield (g m⁻²), total soluble solids (°Brix), titratable acidity (%), ascorbic acid (mg 100⁻¹ ml juice), reducing sugar (%) and non-reducing sugar (%) using the standard procedure.

Statistical Analysis

All the data noted was subjected to *analysis of variance* process to confirm differences among treatments for the studied parameters. Least significant difference (LSD) test was used for mean differences of significant results (Jan *et al.*, 2009).

Results and discussions

Plant height (cm)

Data regarding plant height of strawberry is given in Table 1. Analysis of variance indicated that shade intensity, pre-transplant chilling duration as well as their interaction significantly affected plant height of strawberry. Mean values indicated that daughter plants produced under 70% shade intensity produced taller plants (31.1cm), while shorter plants (16.9cm)

were noted in control treatment where daughter plants were produced under open field. In case of pre transplant chilling duration maximum plant height (28.6cm) was recorded in plots treated with 6 days chilling which was statistically at par with (27.7cm) reported in plants which received 9 days pre transplant chilling duration.

Interaction data for shade intensity and chilling duration revealed that taller plants (33.7cm) were recorded under 70% shade which received 6 days pre transplant chilling, while smaller plants (14.5cm) were observed in non-chilled plants in open field as presented in Fig. 1. Maximum plant height under 70% shade intensity might be due to apical dominance of the plant as a response to avoid shade stress (Moniruzzaman *et al.*, 2009). Similarly Taiz and Zeiger (2010) also reported etiolated growth in plants under low light intensity. The taller plants observed under higher shade indicates a phototropism response in order to help the plants receive enough light (Mapes *et al.*, 2014; Rezai *et al.*, 2018). This increase in plant height under shade might also be due to increase in auxin transportation which leads to cell elongation below the zone of apical meristem leading to increased plant height (Keuskamp *et al.*, 2010).

Literature supports our results by presenting maximum plant height under low light (Haque *et al.*, 2009; Rajasekar *et al.*, 2013). Pre-transplant chilling has great impact on plant canopy architecture (Heide, 1994). In case of chilling durations, plant height of strawberry increased up to the treatment of 6 days pre transplant chilling which might be due to the early sprouting of strawberry plant (Silva, 1982) associated with the fact that, at early phase of development plant depends on its own reserve food for canopy and root development, that will later be an important source of reserve and nutrient exportation that translates into fast growth (Magalhaes, 1985). Similar results are also obtained for other strawberry cultivars by Yamasaki, (2013) and Ledesma *et al.* (2017). Further support to our results is provided by Seno *et al.* (1993) who reported maximum plant height in garlic with increase in vernalization.

Table 1. Plant height, number of crowns plant⁻¹, crown diameter, single fruit weight and total yield of strawberry as affected by shade intensities and pre-transplant chilling durations.

Treatments	Plant height (cm)	Number of crowns (daughter plants) plant ⁻¹	Crown diameter (mm)	Single fruit weight (g)	Total yield (g m ⁻²)
Shade intensity					
0	16.9 c	18.1 c	9.5 a	12.6 a	196.7 a
30%	28.6 b	32.6 a	9.6 a	11.9 b	180.2 ab
50%	29.5 b	27.0 b	9.2 ab	10.9 c	166.2 b
70%	31.1 a	22.1 bc	8.7 b	9.78 d	130.9 c
LSD value (P≤0.05)	1.59	4.89	0.548	0.237	18.30
Chilling duration					
0 days	24.0 c	20.6 b	8.8 c	9.9 c	142.3 c
3 days	25.8 b	26.6 a	9.1 bc	10.8 b	168.3 b
6 days	28.6 a	26.4 a	9.4 ab	12.5 a	186.9 a
9 days	27.7 a	27.3 a	9.8 a	11.9 a	176.3 ab
LSD value (P≤0.05)	0.786	4.88	0.527	0.602	15.58
Interaction (SI x CD)	Fig. 1	NS	NS	NS	NS

Means of the same letter in column are not differ significantly from one another and NS = Non-significant at P≤0.05.

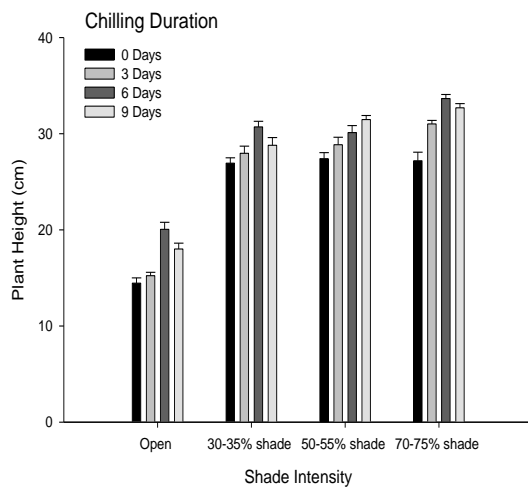


Fig. 1. Interactive effect of chilling duration and shade intensity on strawberry plant height (cm).

Number of crowns plant⁻¹

Statistical analysis of the data indicated that shade levels as well as pre transplant chilling durations significantly affected number of crowns plant⁻¹ of strawberry (Table 1). Number of crowns plant⁻¹ increased with increased in shade level up to 30% and then decrease with further increase in shade intensity. Maximum crowns (32.6) were counted in plants under 30% shade level, whereas fewer leaves (18.1) were noted in plants under open field condition. In case of pre transplant chilling duration more crowns (27.3) were produced by plants received 9 days pre transplant chilling duration which was statistically at par with number of crowns (26.4 and 25.6) produced

in plants subjected to 6 and 3 days pre-transplant chilling duration respectively. While fewer crowns (20.6) were counted in case of non-chilled plants. Photosynthesis; being the main factor for plant growth could be the reason for maximum and minimum crowns plant⁻¹ under 30% and 70% shade level respectively (Raza *et al.*, 2018). In our study minimum photosynthetic pigments were noted in open field due to high temperature. High temperature degrade photosynthetic pigments which ultimately reduces photosynthesis and reparation rate (Todorov *et al.*, 2003; Sharkey and Zhang, 2010). Decline in photosynthesis results in limited resource availability for plant growth and development and thus lead to low plant growth (Young *et al.*, 2004; Sumesh *et al.*, 2008). Moreover decrease in crowns under maximum shade intensity might be due to the low light availability under high shade.

Literature shows that plant growth is directly associated with light intensity (Kiniry *et al.*, 2004) and any reductions in light decreased the plant growth (Maddonna and Otegui, 2004). Optimum light availability enhances plant growth by uplifting physiological and biochemical processes of the plants through capturing and utilizing maximum sunlight which leads to vigorous plant growth. Liu *et al.*, 2015). The low temperature under 30% shade might have increased crowns production (Sharma *et al.*, 2013).

Our results are in conformity with that of Sharma *et al.* (2013) who reported a smaller number of crowns under high temperature. Further support of our result presented in literature that shade level reduces stomatal conductance, photosynthesis rate, carbon assimilation and ultimately plant growth (Kumar *et al.*, 2013; Rezai, *et al.*, 2018). Maximum count for crowns for 6 days pre transplant chilling duration might be due to the cultivar effect used in our study as it is a low chilled cultivar. Also pre transplant chilling helps the plant distribution of sugars during chilling duration in crowns which enhances vegetative growth (Gagnon *et al.*, 1990). Our results are supported by Lieten (2006); Yamasaki, (2013) and Ledesma *et al.* (2017) who reported maximum growth in strawberry with pre transplant chilling.

Crown diameter (mm)

Shade intensity and pre transplant chilling duration significantly affected crown diameter of strawberry as reported in Table 1. Maximum crown diameter (9.6mm) was recorded in plots supplied with 30% shade level which is statistically at par with crown diameter (9.2mm and 9.5mm) of plants grown under 50% shade level as well as open field conditions respectively, while minimum crown diameter (8.7mm) was achieved in plants produced under 70% shade level. In case of pre transplant chilling duration, higher value for crown diameter (9.8mm) was noted in treatment of 9 days chilling duration which was statistically at par with 6 days pre-transplant chilling duration. Whereas non-chilled plants gave lower value (8.8mm) for crown diameter of strawberry. Minimum crown diameter under maximum shade level might be due to the reason that shade reduce plant canopy and enhance vertical growth by distributing assimilated carbon towards it in order to capture maximum light energy (King, 1990; Wu and Tan, 2002). Shading reduces plant diameter while favoring upward growth (Kurepin *et al.*, 2007; Gommers *et al.*, 2013; Yang *et al.*, 2014). Our results are in similarity with Sharma *et al.* (2013) who reported maximum crown diameter under low shade level. Maximum crown diameter with maximum pre transplant chilling duration might be

due to maximum leaves which enhances photosynthesis. Maximum photosynthesis improves photosynthate formation in plant which is further transferred from leaves to the sink organ. Thus maximum photosynthates in the sink organ leads to maximum plant diameter (Long *et al.*, 2004; 2006). Our results are in line with Lefebvre *et al.* (2005) who reported maximum growth in tobacco due to maximum photosynthate accumulation.

Single fruit weight (g)

Table 1 represents data pertaining single fruit weight of strawberry. It is evident from analysis of variance that shade intensity and chilling duration exhibited significant variation in single fruit weight of strawberry. No application of shade i.e. open field produced weightier fruits (12.6g), while lighter fruits (9.8g) were noted under 70% shade level. Similar increase in single fruit weight of strawberry was observed with increasing chilling duration. Single fruit weight was maximum (12.5g) in plants which received 6 days chilling which is statistically at par with (11.9g) in plants which received 9 days pre-transplant chilling, while it was minimum in control treatment (9.9g). Single fruit weight is major factor contributing to the total yield of strawberry. Although fruit weight is predominantly a genetic character, yet it totally depends upon different growth parameters (Asma, 2011). Shade application negatively affect fruit weight as observed in our study which might be due to low photosynthesis under shade. Reduced photosynthesis caused stomatal closure which leads to low gas exchange and carbon assimilation thus lead to reduced fruit weight. Our results are supported by the reports of Fletcher *et al.* (2002) and Ozturk *et al.* (2004) who reported fruit weight reduction due to shade. Fruit weight is totally dependent upon the food supply to the fruit which is directly related to number of leaves as well as leaf area of the plant (Zhu *et al.*, 2010). This could be the possible reason of maximum fruit weight with 6 days pre transplant chilling duration as in our study we observed maximum leaf count as well as area with 6 days pre transplant chilling duration. Our results are supported by Mathan, *et al.* (2016) who reported maximum fruit weight with increase in leaves.

Total yield (g m⁻²)

It is evident from the analysis of variance that shade levels as well as chilling durations had significant while their interaction had non-significant effect on the yield of strawberry fruit (Table 1). The mean values indicated that maximum yield (196.7g m⁻²) was achieved by the control treatment, while minimum yield (130.9g m⁻²) were noted in plots which received 70% shade. In case of pre transplant chilling duration maximum total yield (186.9g m⁻²) was recorded in plots received 6 days chilling, while the lowest total yield (142.3g m⁻²) was recorded in non-chilled plants. Maximum fruit yield in open field might be due to adequate light availability. Light increase total yield by improving photosynthesis and delaying leaf senescence (Hovi *et al.*, 2004; Hovi and Tahvonon 2008). Negative effect of shade on total fruit yield as presented in our study might be due to low fruit set caused by stamen abortion in low light (Johannes, 2008). Reduction in total yield due to shade is further supported by Ibrahim *et al.* (1997). Yield reduction under shade in strawberry is closely related to reduced photosynthesis in the plants due to unavailability of sufficient light (Choi *et al.*, 2016). Fruit yield is directly related with vegetative growth of the plant. Thus maximum fruit yield with 6 days pre transplant chilling duration might be due to the maximum single fruit weight as well as leaves count and area at this level. Leaves are the primary site of photosynthesis, thus plants with maximum number

of leaves as well as leaf area efficiently harvest light, leading to a faster growth rate and increased yield (Zhu *et al.*, 2010). Our results are supported by Mathan, *et al.* (2016) by reporting maximum yield with increase in leaves.

Total soluble solids (°brix)

Statistical analysis of the data indicated that shade levels significantly affected total soluble solids of strawberry (Table 2). More soluble solids (10.3°brix) were measured in plots treated with no shade followed by 30% shade level, whereas lesser amount of total soluble solids (7.0°brix) was noted in the strawberry of plots which received 70% shade. For proper plant growth and developmental processes soluble sugars are very necessary. They not only provide energy to the plant but also signalize and regulate important movements of plants (Wang and Tang, 2014). Sugar content along with other factors are much dependent on growing condition (Hamano *et al.*, 2002). Maximum total soluble solids in open field might be due to more leaves in open field as according to Carlen *et al.* (2007). Decrease in total soluble solids with effect of shade might be due to low light stress caused by shade net (Morgan, 2006). Sen *et al.* (2012) also reported higher TSS in grapes under open field conditions. In case of pre transplant chilling duration total soluble solids didn't seem to be effected significantly and this result is supported by the findings of Oviedo *et al.* (2018).

Table 2. Total soluble solids, titratable acidity, ascorbic acid, reducing sugar and non-reducing sugar of strawberry as affected by shade intensities and pre-transplant chilling durations.

Treatments	Total soluble solids (°brix)	Titratable acidity (%)	Ascorbic acid (mg 100 ml ⁻¹)	Reducing sugar (%)	Non reducing sugar (%)
Shade intensity					
0	10.3 a	1.0 a	56.3 a	5.0 a	1.7 a
30%	8.9 b	0.9 ab	54.0 b	4.7 ab	1.6 ab
50%	7.9 c	0.9 bc	46.5 c	4.2 c	1.5 bc
70%	7.0 d	0.80 c	43.1 d	3.5 bc	1.4 c
LSD value (P≤0.05)	0.351	0.084	1.267	0.441	0.177
Chilling duration					
0 days	8.2	0.9	47.9	4.2	1.4
3 days	8.6	0.9	49.6	4.6	1.5
6 days	8.8	1.0	52.3	4.8	1.7
9 days	8.4	0.9	50.0	4.7	1.6
LSD value (P≤0.05)	NS	NS	NS	NS	NS
Interaction (SI x CD)	NS	NS	NS	NS	NS

Means of the same letter in column are not differ significantly from one another and NS = Non-significant at P≤0.05.

Titrateable acidity (%)

Statistical analysis of the data indicated that shade intensities has significantly while chilling duration as well as the interaction between shade intensity and chilling duration has non-significantly affected titrateable acidity of strawberry (Table 2). Titrateable acidity was maximum (1.0%) in fruits under control treatment and decreased with increasing shade levels. Minimum titrateable acidity (0.8%) was noted in fruits under 70% shade intensity. Higher titrateable acidity was observed in fruits grown without shade which might be due to high light intensity (Haselgrove, *et al.*, 2000). Light favors quality enhancement due to synthesis of some plant hormones (Watson *et al.*, 2002) or because of effects on the temperature of the fruit. TA increase with increase in light intensity (Anagnostou and Vasilakakis, 1995). Contrary to our results, Saidi *et al.* (2013) reported lower TA in tomato grown under nets compared with the no net.

Ascorbic acid (mg 100 ml⁻¹)

Significant effect of shade level while non-significant effect of chilling duration as well as the interaction between shade level and chilling duration on ascorbic acid of strawberry is reported in Table 2. Maximum ascorbic acid (56.3mg 100ml⁻¹) was recorded in the treatment of open field, while 70% shade resulted minimum ascorbic acid (43.1mg 100ml⁻¹). Sourness and taste of fruit is regulated by ascorbic acid (Asma, 2011). It is regulated by both biotic as well as abiotic factors (Lopez-Marin *et al.*, 2011, 2012). Maximum ascorbic acid in open field might be due to maximum light availability in open field. Light favors ascorbic acid accumulation (Dumas *et al.*, 2003), while shade reduces it (Liu *et al.*, 2004). Decrease in fruit ascorbic acid under shade might be due to low sugar levels (Nishikawa *et al.*, 2005) as the sucrose regulates metabolism of ascorbic acid precursor in chloroplast. Thus sugar levels may act as a signal (Smeekens, 2000) and may consequently affect ascorbic acid precursor and ultimately ascorbic acid content in fruit. Our results are supported by and Milenkovic *et al.* (2012) who reported maximum ascorbic acid content in unshaded fruits.

Reducing sugar (%)

Shade levels significantly affected reducing sugar of strawberry (Table 2). The chilling duration and interactive effect of SL and CD was non-significant. Mean values of the data for shade intensities indicated that no shade resulted maximum reducing sugar (5.0%), while minimum reducing sugar (3.5%) was recorded in case of 70% shade intensity. Reducing sugar in strawberry is negatively affected by shade levels. Maximum reducing sugars in open field conditions might be due to the maximum amount of TSS in open field as total soluble solids in strawberry comprise more than 65% of reducing sugar such as fructose, glucose and sucrose (Wang and Lin, 2002). Our results are supported by Abeysinghe *et al.*, 2019 who reported less sugar content in shaded berries. Callejon-Ferre *et al.* (2009) showed sugar reduction in tomato fruit content with increase in shade density.

Non-reducing sugar (%)

Statistically analyzed data revealed that non reducing sugar in strawberry significantly varied by various shade levels (Table 2). Chilling duration and interactive effect of shade levels and chilling duration was found non-significant. Mean values indicated that increasing shade level decreased the amount of non-reducing sugar in strawberry. Fruit production in open field resulted in maximum non reducing sugar (1.7%), while minimum non reducing sugar (1.4%) was recorded in case of 70% shade level. Maximum non-reducing sugar in strawberry under open field conditions might be due to the maximum amount of TSS in open field plants (Wang and Lin, 2002). Shade effects plant carbon balance of the plants as low light enhances the physiological processes decreases while photosynthetic rate (Yang *et al.*, 2017). Support to our study is provided by Su *et al.* (2014) and Yang *et al.* (2017) who reported minimum photosynthesis and maximum assimilate demand in soybean with increase in shade. Our results are further supported by Abeysinghe *et al.* (2019) who reported less sugar content in shaded berries.

Conclusion

It is concluded from the results that for increase in daughter plants production, strawberry cultivation should be done under 30% shade intensity with

subjection to 6-9 days pre transplant chilling, while for qualitative as well as quantitative fruit increase, open field along with 6 days pre-transplant chilling duration is recommended for the agro climatic condition of Swat, Khyber Pakhtunkhwa-Pakistan.

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