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Effect of Different Spacing and Varieties on Growth, Quality and Yield in Onion (*Allium cepa* L.)

Braj Kishor^a, M. L. Meena^b, Nirankar^{a*}, Subash Verma^c, Ankit Kumar Goyal^a, Shani Kumar^d and K. S. Yadav^e

^a Department of Vegetable Science, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, (U.P.)-208 002, India.

^b Department of Horticulture, School of Agricultural Sciences and Technology, Babasaheb Bhimrao Ambedkar University (A Central University) Vidya Vihar, RaeBareli Road, Lucknow, U.P.,-226025 India.

^c Department of Vegetable Science, Bihar Agriculture University Sabour, Bhagalpur (Bihar)-813210, India.

^d Department of Vegetable Science, Acharya Narendra Deva University of Agriculture and Technology, Kumargang, Ayodhya, (U.P.)–224229, India.

^e Department of Fruit Science, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, (U.P.)-208 002, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present experiment was conducted at the field of Horticulture Research Farm, Babasaheb Bhimrao Ambedkar University, Lucknow (Uttar Pradesh) during *rabi* season of 2019-2020. The experiment was laid out in factorial randomized block design (FRBCD) and treatments comprised

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^{*}Corresponding author: E-mail: nirankarverma437@gmail.com, nirankarv233@gmail.com;

of three varieties *i.e.* V₁ (Agrifound Light Red), V₂ (NHRDF-Red), V₃ (NHRDF-2) and three planting spacing S₁ (20×7.5cm), S₂ (20×10cm) and S₃ (20×15 cm). The parameters were observed *viz.* plant height (cm), number of leaves /plants, neck thickness (cm), bulb diameter (cm), Number of scales, bulb length (cm). TSS (°brix), ascorbic acid (mg/100gm), total sugar (%), reducing sugar (%), non-reducing sugar (%), acidity (%) yield per plot (kg) and yield per hectare (q). The results of this study revealed that the combination application of variety (Agrifound Light Red (V₁) with spacing 20X 15 cm (S₃) was best under Lucknow conditions and lowest values were noted in of variety Agrifound Light Red (V₁) with spacing 20X 7.5 cm (S₁).

Keywords: Acidity; ascorbic acid, TSS; yield.

1. INTRODUCTION

Onion (Allium cepa L., 2n=16) is very common vegetable of Rabi season crop belongs to alliaceae family. It is also regarded as aueen of vegetable [1]. This crop is native to Central Asia. Onion is grown throughout the entire world with some major producing countries like China, India, USA, Turkey, Pakistan, Iran, Japan, Spain and Brazil. In the world, India stands first in area (1293 Mha.) and ranks second in production (23610 M tons) after China. India exported fresh onion worth \$328 million and dried onions worth \$112.3 million in Financial year 2020. Export of onion shot up 158% to Bangladesh in the April-July period of 2019-20 (ministry of commerce & industry, Government of India). The crop is very useful for human being because it has several nutritional and medicinal values. The important contents like allicin, allin, and sulphites etc. are present in onion in good amount. Onion has antianti-bacterial, viral. anti-allergenic, antiinflammatory properties and due to the presence of flavonoids it reduces the chance of cancer and diabetes [2-4]. The antifungal property of onion is due to presence of catechol a phenolic compound. Onion is used for treating problems including loss of appetite, upset stomach, and gall bladder disorder, for treating heart and blood vessel problems including chest pain (angina). It also acts as a very good tonic to a nervous system and purifies blood. The beneficial compound called 'Quercetin' present in onion has shown to be powerful antioxidant [5-7]. It pungency is due to the presence of Allyl propyl disulphide, $(C_6 H_{12}O_2)$ a volatile oil. The yield of vegetable crops can be increased by choosing a suitable cultivar, judicious use of manures and fertilizers with proper spacing, adequate water supply and cultural practices etc [8,9]. Therefore, the use of proper geometry to get appropriate plant stand is pre- requisite for higher crop yield per unit area.

2. MATERIALS AND METHODS

A field experiment was conducted durina the rabi season of October to April 2019-20 at Horticulture Research Farm. School of Agricultural Sciences and Technology Babasaheb Bhimrao Ambedkar University (A Central University), Vidya- Vihar Rae Bareli Road, Lucknow,. The experimental field was undertaken is geographically, situated at an elevation of 111 meter above mean sea level in the sub- tropical climate of central Utter Pradesh at 26°56 North Latitude 80°52 east longitude. According to agroclimatic conditions of Uttar Pradesh state, Lucknow falls under central plains region and receives on an average 1000 mm of Rainfall; the climate ranges from 22°C to 45°C in summer, minimum temperature ranging from 3.5 -15°C in winter and relative humidity ranging from 60-80% in different season of the year. The soil of field was texturally classified as sandy clay loam and slightly alkaline in reaction. The treatments comprised of three varieties i.e. Agrifound Light Red, NHRDF-2 NHRDF-Red, and three planting spacing (20x7.5cm, 20x10cm and 20x15 cm) thus forming nine treatments were laid out in a factorial combinations randomized block design of practices. Observations to be recorded growth characters viz. Plant height (cm), Number of leaves /plants, Neck thickness (cm); bulb characters viz. Bulb diameter (cm), No. of scales, Bulb length (cm); quality characters viz. TSS (°Brix), Ascorbic Acid (mg); Total Sugar (%), Reducing sugar(%), non- reducing sugar(%) and Acidity(%) and yield parameters viz. yield per plot (kg / ha) and yield per hectare (q/ha). Statistical analysis of data was obtained in different set of experiments was calculated following the standard procedure as stated by Panse and Sukhatme.

3. RESULTS AND DISCUSSION

3.1 Growth Characters

3.1.1 Plant height

The plant height was recorded at 30, 60, 90 DAT and at harvest stage. The data showed that there was significant difference among varieties and spacing on plant height. The Data presented in Table 1, clearly indicate that at 30DAT, Variety V₃ (NHRDF-2) had highest plant height (16.90cm) followed by variety V_2 (NHRDF-Red) = 16.48cm, at 60 DAT variety V2 (NHRDF-Red) had maximum plant height (43.69cm) followed by V_3 (NHRDF-2) =40.87 cm, 90 DAT Variety V_3 (NHRDF-2) had maximum plant height (65.33 cm) followed by V_2 (NHRDF- Red) = 63.29 cm and at harvest stage variety V₃ (NHRDF-2) produce maximum height (65.33cm) followed by variety V_1 (Agrifound Light Red) = 64.77cm . In case of spacing, there were also significant variations in observations. At all stages (30, 60, 90 DAT and harvesting) maximum plant height was recorded with the spacing of S_3 (20X 15cm), 17.65, 45.34, 65.68 and 67.15 cm followed by S₂ (20X10cm); 16.58, 39.70, 61.85 and 63.64cm respectively. The combined effect of varieties and plant spacing also revealed that there was significant variation on plant height at all stages of plants growth. Among the interactions, at the all stages (30, 60, 90 and at harvesting), the maximum plant height was recorded under V_1S_3 ; 18.95, 48.55, 68.02, 69.53 cm followed by V_3S_2 . Similar result was reported by Jilani et al. [10].

3.1.2 Number of leaves

The number of leaves/plant were counted at 30. 60, 90 and harvest stage. The data was recorded is presented in the Table 1 indicate that there was significant change in varieties and spacing on number of leaves / plant at all the stages of growth. The data revealed that at 30 DAT highest number of leaves /plant (3.49) were recorded in V₃ (NHRDF- 2) followed by variety V₁ Agrifound Light Red (3.42), at 60 DAT maximum number of leaves / plant (4.37) were in variety V₃ NHRDF-2 followed by V₁ Agrifound Light Red (4.34) and 90 DAT maximum number of leaves were in variety NHRDF-2 (6.38) followed by V1 Agrifound Light Red (6.18) and at harvest V1 (Agrifound Light Red) produce maximum number of leaves / plant (10.37) followed by variety V_3 NHRDF-2 (10.18). In case of spacing, It was recorded that the plant spacing S_1 (20×15cm) produce the maximum number of leaves/plant (3.48) followed by (3.44) at 30 DAT, the plant spacing S_3 (20×15cm) produce the maximum number of leaves /plant (4.36) followed by S1 (20×7.5cm) (4.35) at 60 DAT, the plant spacing S_3 (20X15) produce maximum 6.50 leaves per plant followed by S₂ (20X10)= 6.04 at 90 DAT and the spacing S_3 (20×15 cm) produce maximum number of leaves/plant (11.13) followed by S_2 (20x15cm) =9.88 at harvest. The combination effect of the variety and plant spacing was also found significant effect on number of leaves /plant at all stages of plant growth. Among interactions, the maximum number of leaves/plant (3.64, 4.54, 6.54 and 9.54) were recorded in V₁S₃ followed by V2S1 (3.55, 4.45, 6.45 and 9.45) at all stages respectively. These findings were closely related with the result of Devi et al. [11].

3.1.3 Neck thickness

The data regarding to neck thickness (cm) was recorded at 30, 60, 90 DAT and at harvest. The data presented in Table 1 shows significant variation of varieties and spacing on neck thickness. Data shows that highest neck thickness (0.427, 0.713, 1.996 and 2.121 cm) as reported in variety Agrifound Light red followed by variety NHRDF-2 (0.334, 0.567, 1.883 and 2.114cm) respectively.

It was noticed that plant spacing was also significantly affected by Varieties. Plant spacing S₂ (20×10cm) showed maximum neck thickness (0.388 cm) followed by S₃ $(20 \times 15 \text{ cm}) = 0.352 \text{ cm}$ at 30DAT, spacing S₁ (20×15 cm) produce maximum neck thickness (0.633cm) followed by S_2 (20×10cm) = 0.578cm at 60 DAT, maximum neck diameter was recorded in S_3 (20X15) = 1.98 followed by S₁ (20 X 7.5) =1.91 at 90 DAT and plant spacing S₃ (20×15cm) recorded maximum neck thickness (2.141cm) followed by S_2 (20×10 cm)= 2.107cm at harvest. The combined effect of variety and plant spacing also showed that there was significant change on neck thickness at all stages of plants growth. Among the interactions, the maximum neck thickness was observed in V_1S_2 (0.513cm) followed by the V_1S_3 (0.420cm) at 30 DAT, maximum thickness (0.853, 2.043, 2.157cm) at 60, 90 DAT and harvest was noted in V₁S₃ followed by V_1S_1 (0.673, 1.990 and 2.107cm). The findings are closely related with Kumar et al. [12].

3.2 Bulb Characters

3.2.1 Bulb diameter

The data regarding to Bulb diameter of onion is presented in Table 1. After perusal of the data, it

is clear that maximum bulb diameter (6.86cm) was noted in Variety V₂= NHRDF- Red followed by $V_3 = NHRDF-2$ (6.86 cm) and minimum in $V_1 =$ Agrifound Light Red (5.69cm) respectively. The effect of the plant spacing on bulb diameter (cm) was also found significant and maximum diameter was recorded in S₂ 20x10cm =7.14cm followed by S_1 (20X10cm) = 6.20cm and minimum diameter was found in S3 (20x15cm) = 6.18cm. The combined effect of varieties and spacing on bulb diameter (cm) was also found significant and maximum diameter was found in $V_1S_3 = 7.45$ cm followed by V_2S_1 7.45 cm), V_1S_2 (6.57 cm), V_2S_2 (5.69cm) and minimum diameter was found in V_1S_1 (4.80cm). Similar findings were also noticed by Khan et al. [13]; and Jilani et al. [10].

3.2.2 Bulb length

The data regarding to bulb length of onion has is showed in Table 1. After perusal of the data, it is clear from the table that highest bulb length was noted in variety V_2 NHRDF-Red = (5.29 cm) followed by V_3 : NHRDF-2, = 5.10cm while lowest bulb length (cm) was found in variety V_1 (Agrifound Light Red) = 4.86cm. The effect of the plant spacing on bulb length was also found significant and maximum bulb length was found at S_2 (20x10cm) = (5.72cm) and it was found minimum in S_1 (20x7.5cm) = 4.69. The combined effect of plant varieties and spacing on bulb length (cm) was also found significant and maximum length was found in V_2S_2 (6.53cm) and minimum length was found in V_1S_1 as (4.42cm). This result corroborated the finding of Jilani et al. [10].

3.2.3 Number of scales

The data regarding to number of scales of bulb is presented in Table 1. It is evident from the data that highest number of scales (11.67) was noted in variety V₃ (NHRDF- 2), while lowest number of scales (10.11) reported in variety V₂ (NHRDF-Red). The effect of the plant spacing on number of scales was also found significant and maximum scales were found at S_3 (20x15cm) =11.89 and lowest number of leaves was found in S2 (20x10cm) = 10.22. The combined effect of plant varieties and spacing on number of scales was also found to be significant and maximum scales were found in V_1S_3 (12.33) and minimum number leaves was found in V_1S_2 (9.00). These results are close conformity with earlier reports of Jilani et al. [10].

3.3 Quality Parameters

3.3.1 TSS

An inquisition of data in Table 2 clearly indicate that among the varieties maximum TSS (11.71° brix) was recorded in variety V₁ (Agrofound Light Red) followed by V₃ (NHRDF-2= 1.18°brix. and the minimum TSS (10.80°brix) was noted of variety V₂ (NHRDF-Red).The maximum TSS content (11.45°brix) was noted as plant spacing S₂ (20x10cm) while the minimum TSS content (10.89°brix) observed under plant spacing S₁ (20x7.5cm). The combined effect of varieties and plant spacing showed significant influence on TSS content in different onion varieties spacing. Among the treatments (V₁S₃) recorded highest TSS (12.80°brix).

3.3.2 Ascorbic acid

The findings of the ascorbic acid of present experiment is showed in Table 2 which showed significant variation in variety and plant spacing on ascorbic acid content. It is clear from the data that variety V₃ (NHRDF-2) contain highest ascorbic acid content (0.74mg /100g) followed by V₂ (0.70mg/100g) and the lowest ascorbic acid content was noted in V₁ (0.69mg/100g). Among the plant spacing maximum ascorbic acid was determine with S_2 (20X10) = 0.76mg/100g followed by S_3 (20x15cm) = 0.74mg/100g whereas minimum ascorbic acid content as (0.64mg/100g) was noted under plant spacing S₁ (0.63 mg/100 g). The combined effect of variety and plant spacing also revealed statistically significant influence on ascorbic acid content. Among the combination V₁S₃ recorded highest (0.84mg/100g) ascorbic acid and minimum was noted in V_1S_1 (0.42mg/100g).

3.3.3 Reducing sugar

The data regarding to reducing sugar presented in the Table 2. and showed significant effect of variety and plant spacing was found on reducing sugar content in bulb. Highest reducing sugar content (7.56%) was found in V₂ followed by V₁ (6.67%) while The lowest reducing sugar content (6.61%) was noted in V₃. Among the plant spacing maximum reducing sugar (7.26%) was recorded with S₂(20x10cm) followed by S₃ (20x15cm,7.25%) whereas The minimum reducing sugar content (5.50%) was noted under plant spacing S₁(20x7.5cm). The combined

Treatment					Plant Height		Number of Leaves (cm)			Neck thickness (cm)				Number of scales	Bulb height (cm)
	30DAT	•	60 DAT	90 DAT	At harvest	30 DAT	60 DAT	90 DAT	At harves	st 30 DAT	60 DAT	90DAT	At harvest		(0)
							Effe	ct of Varie	ties						
Agrifound Ligh	nt 16.06	39.84	62.96	64.7	3.42	4.34	6.18	10.37	0.427	0.713	1.996	2.121	5.69	10.66	4.86
NHRDF-Red	16.48	43.69	63.29	62.63	3.39	4.27	5.95	10.12	0.327	0.506	1.839	2.058	6.97	10.1	5.29
(V_2) NHRDF -2	16.91	40.87	65.33	65.33	3.49	4.37	6.38	10.18	0.334	0.567	1.883	2.114	6.86	11.67	5.10
SEm(±) CD (P=0.05)	0.19 0.57	0.33 1.02	0.16 0.48	0.16 0.48	0.021 0.06	0.02 0.07	0.02 0.07	0.02 0.07	0.01 0.03	0.03 0.09	0.02 0.09	0.03 0.10	0.31 0.99	0.23 0.69	0.27 0.83
(Effe	ct of Spac	ing						
20×7.5 (S ₁)	15.21	39.35	60.37	61.95	3.48	4 .26	5.97	9.66	0.348	0.673		2.046	6.20	10.33	4.69
20×10 (S ₂)	16.58	39.70	61.85	63.64	3.38	4.27	6.04	9.88	0.388	0.578	1.817	2.107	7.14	10.22	5.72
20×15 (S ₃)	17.65	45.34	65.68	67.15	3.44	4.35	6.50	11.13	0.352	0.633	1.989	2.141	6.18	11.89	4.84
SEm(±)	0.19	0.33	0.35	0.16	0.02	0.02	0.02	0.02	0.012	0.032	0.025	0.036	0.312	0.23	0.27
CD(5%)	0.56	1.02	0.10	0.48	0.06	0.07	0.07	0.07	0.038	0.097	0.077	0.106	0.993	0.69	0.83
							Effec	t of intera	ction						
$V_1 S_1$	14.29	35.64	58.36	59.83	3.33	4.23	6.23	9.22	0.347	0.673		2.107	4.80	10.67	4.42
$V_1 S_2$	14.95	35.32	62.49	64.97	3.34	4.24	6.24	9.24	0.513	0.613	1.953	2.080	6.57	62.24	4.83
V_1S_3	18.95	48.55	68.02	69.53	3.64	444.00	6.54	9.54	0.420	0.853	2.043	2.157	7.45	65.67	5.81
V_2S_1	14.70	42.36	64.45	60.47	3.55	4.45	6.45	9.45	0.330	0.470	1.713	1.920	7.08	58.31	4.61
$V_2 S_2$	16.46	43.00	62.51	61. 78	3.29	4.19	6.19	9.20	0.317	0.503	1.853	2.110	5.69	59.89	6.53
V_2S_3	18.28	45.69	63.29	65.62	3.29	4.19	6.19	9.19	0.330	0.543	1.950	2.143	6.38	58.03	4.74
V_3S_1	16.64	40.04	58.30	66.29	3.50	4.34	6.40	9.40	0.367	0.580	2.033	2.110	6.71	64.26	5.03
V_3S_2	18.34	40.78	60.56	64.15	3.48	4.38	6.38	9.38	0.333	0.617	1.643	2.130	7.39	59.17	5.32
V_3S_3	15.73	41.77	65.73	65.55	3.51	4.34	6.34	9.34	0.303	0.503	1.973	2.123	6.48	61.87	4.47
SEm(±)	0.33	0.58	0.61	0.28	0.037	0.42	0.42	0.43	0.022	0.055	0.044	0.063	0.54	1.10	0.47
CD (5%)	0.99	1.77	1.85	0.84	0.01	1.56	0.12	0.13	0.064	0.162	0.134	0185	1.59	3.34	1.4

Table 1. Treatment wise variation in plant height, leaf and bulb numbers

Treatment	TSS	Ascorbic Acid	Total Suger	Reducing	Non Reducing	Acidity (%)	Yield per	Yield per		
	(Brix)	(mg)	(%)	Suger (%)	Suger (%)		plot (kg)	hectare (q)		
				Effect of Varieties						
Agrifound light	11.17	0.69	16.13	6.71	8.79	0.039	4.10	331.41		
Red (V ₁)										
NHRDF-Red (V ₂)	10.80	0.71	17.05	7.59	8.51	0.037	3.25	262.16		
NHRDF -2 (V_3)	11.17	0.74	16.54	6.59	8.78	0.037	3.46	281.48		
SEm±	0.05	0.02	0.07	0.06	0.04	0.003	0.12	9.62		
CD(P=0.05)	0.15	0.06	0.21	0.02	0.14	0.009	0.36	29.10		
Effect of Spacing										
20×7.5 (S ₁)	10.89	0.63	16.27	6.38	8.42	0.031	3.72	301.41		
$20 \times 10 (S_2)$	11.45	0.76	16.87	7.26	8.36	0.041	3.75	298.83		
20×15 (S ₃)	11.33	0.74	16.59	7.25	9.31	0.040	3.35	274.69		
SEm(±)	0.05	0.02	0.07	0.06	0.04	0.003	0.12	9.62		
CD(P=0.05)	0.15	0.06	0.21	0.20	0.13	0.009	0.36	29.10		
				Interaction effect						
V ₁ S ₁	10.29	0.42	14.69	5.50	8.29	0.030	4.12	343.87		
$V_1 S_2$	12.04	0.80	15.52	6.27	8.32	0.040	3.70	284.13		
V_1S_3	12.80	0.84	18.19	8.38	9.76	0.047	4.49	365.85		
V_2S_1	10.08	0.68	17.97	7.24	7.77	0.030	3.12	239.71		
$V_2 S_2$	11.23	0.73	17.08	8.32	8.63	0.040	3.78	307.39		
V_2S_3	11.10	0.70	16.11	7.19	9.13	0.040	2.86	239.37		
V_3S_1	12.32	0.80	16.17	6.40	9.19	0.033	3.94	320.66		
V_3S_2	11.1	0.73	18.01	7.19	8.13	0.043	3.75	304.95		
V_3S_3	10.11	0.68	15.46	6.17	9.02	0.033	2.69	218.83		
SEm(±)	0.08	0.03	0.12	0.11	0.07	0.005	0.20	16.67		
CD (5 %)	0.26	0.11	0.37	0.35	0.23	0.014	0.62	50.40		

 Table 2. Treatment wise variation in total sugar, acidity and yield per plant

effect of the variety and plant spacing revealed statistically significant influence on reducing sugar content. Among the treatment V_1S_3 recorded highest reducing sugar (8.25%) and minimum (5.50%) was noted in V_1S_1 .

3.3.4 Non-reducing sugar

Significant differences were observed among different treatments for non - reducing sugar as presented Table 2. It is clear from data that variety V₁ had highest non-reducing sugar content (8.79%) followed by V_3 (8.78%) while the lowest non- reducing sugar content (8.36%) was noted in V₂. Among the plant spacing maximum non-reducing sugar (9.31%) was determine with S_3 (20x15cm) followed by S_1 (20x7.5cm) = 8.41% whereas the minimum non-reducing sugar content as 8.30% was noted under plant spacing S_1 (20x7.5cm). The combined effect of the variety and plant spacing revealed statistically significant influence on non-reducing sugar content. Among the treatment V_1S_3 recorded highest (9.76%) non reducing sugar and minimum was noted in $V_2S_1 = 7.76\%$.

3.3.5 Total sugar

The data showed significant variation of variety and plant spacing on total sugar content in bulb as presented in Table 2. It is evident from the data that variety V₂ contain highest total sugar contents (17.05%) followed by V₃ (NHRDF-2; 16.54%). The lowest total sugar content was noted in V₁ (Agrifound Light Red; 16.13%). Among the plant spacing, maximum total sugars (16.87%) was determined with S_2 (20x10 cm) followed by S_3 (20x15 cm; 16.59%). The minimum total sugar content (16.27%) was noted under plant spacing S1 (20x7.5cm). The different between S_2 and S_3 are found significant. The combined effect of variety and plant spacing revealed statistically influence on total sugar content. Among the treatment V_1S_3 recorded highest total sugar (18.19%) and minimum (14.69%) was noted in V_1S_1 .

3.3.6 Acidity

As the data revealed from the Table 2. It is clear that variety V_1 had highest acidity content (0.039%) followed by V_3 (0.037%) while the lowest acidity content (0.035%) was found in by V_2 . Among the plant spacing maximum acidity (0.41%) was determined with S_2 (20x10cm) followed by S_3 (20x15cm) = 0.040% while minimum acidity content (0.31%) was noted

under the plant spacing $S_1(20x7.5cm)$. The combined effect of variety and plant spacing revealed statistically significant influence on acidity content. Among the combination V_1S_3 recorded highest acidity (0.047%) and lowest was noted in V_1S_1 (0.030%).

3.4 Yield Parameters

3.4.1 Yield per plot

The data recorded on yield per plot is given in Table 2. it is clear from the table that highest yield (4.102 kg) was recorded in variety V₁ (Agrifound Light Red), while lowest yield (3.25kg) in variety V₂ (NHRDF-Red).The effect of plant spacing on yield per plot was found significant and h highest yield (3.72kg) was found at S₁ (20x7.5cm) whereas lowest yield (3.35 kg) was found in S₃ (20x15cm). The combined effect of plant varieties and spacing on yield per plot (kg) was also found significant and maximum yield (4.49kg) was found at V₁S₃ and minimum yield (2.69kg) obtained in V₃S₃.

3.4.2 Yield per hectare

A glance of data in Table 2 showed significantly variation by different treatments in yield per hectare. It is clear from the table that highest yield (331.28q/ha) was obtained in variety V₁ (Agrifound Light Red) while lowest (262.15q) was found in variety V₂ (NHRDF-Red). The effect of plant spacing on yield per hectare was also found significant and maximum yield (301.42q) was found at S₁ (20x7.5cm) and lowest yield (274.68q) was observed in S₃ (20x15 cm.). The combined effect of the varieties and spacing on yield per hectare was also found significant and maximum yield (365.85q) was found at V₁S₃ while lowest yield (218.83q) has been found in V₃S.

4. CONCLUSION

From the above results, it can be concluded that variety Agrifound Light Red with spacing 20X 15 producing significantly highest growth, yield and quality bulb compared to other varieties and spacing. The values of growth, yield and quality characters were found to be decreased with the decreasing of plant spacing and finally the minimum values of these parameters were recorded in the closest spacing 20X 7.5cm. The plant grown under wider spacing received more nutrient light and moisture compared to the closer spacing which is the probably reason of better performance and yield of individual onion in wider spacing.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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