

# Effect of Planting Density and Chlormequat Chloride on Morphological and Physiological Characteristics of Winter Barley (*Hordeum vulgare L.*) Cultivar "Valfajr"

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## ABSTRACT

In a field experiment the effect of five planting densities (100, 175, 250, 325 and 400 plants m<sup>-2</sup>) with or without foliar application of chlormequat chloride (2-till o methyl-trimethyl ammonium chloride, CCC) on **growth**, development and grain yield of winter barley (cv Valfajr) was studied. The results indicated that **Increasing** plant density hastened the rate of apical development and stem elongation. This pattern continued up to the beginning of rapid stem elongation phase (Zadoks growth stage 32). However, during the rapid stem elongation and ear growth, the pattern was reversed i.e. it had become slightly in favor of the lower densities. Increasing plant density up to 250 plants m<sup>-2</sup> increased the grain yield, however, from 250 to 400 plants m<sup>-2</sup> there was no significant change in grain yield. It appeared that the higher planting densities, i.e. 250 to 400 plants m<sup>-2</sup> were in a range around the supposed "optimum" density. Foliar application of CCC slowed down the rate of apical development and stem elongation in all plant densities, but without any significant effect on its spikelet initiation rate. Such retardation of development in CCC-treated plants was initially associated with lower dry weight accumulation. However, this trend was reversed before anthesis, as the CCC-treated plants had accumulated more dry matter by anthesis, and gave a higher grain yield. The interactive effect of CCC and plant density is worthy of further exploration.

Keywords: Yield components, Planting density, Growth regulation, Plant development.

## INTRODUCTION

The success of chlormequat chloride (2-chloroethyl-trimethyl ammonium chloride, CCC) in reducing cereal crops height, thereby reducing or preventing lodging ensured its wide commercial use in the late 1960s, i.e. in a period when lodging was perhaps the most critical problem in the wheat crop, (Nafziger *et al*, 1986; Moes and Stobbe, 1991; Khan

and Spilde, 1992; Ma and Smith, 1992 a,b). Although the introduction of semi-dwarf wheats with the *Rht* (reduced height) genes largely solved the problem of lodging, evidence was already accumulating that a timely application of such growth retardants as CCC can increase yield of both wheat and barley, independently of any control on lodging (Pinthus and Rudich, 1967; Bragg *et al* 1984). In barley, a timely application of CCC

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at a prescribed stage of apical development has been shown to increase the grain yield by 10-20% through increasing grain number without any compensatory decrease in grain size (Koranteng and Matthews, 1982, Waddington and Cartwright 1986, Emam and Karimi, 1996). It has been suggested that CCC-treated crops may have a higher optimum population density (Williams *et al* 1982). This suggestion is justified in the light of the knowledge that early stem elongation and apex development are promoted in higher plant densities (Kirby, 1967; Kirby and Faris, 1970) or when GA3 is applied (Kirby and Appleyard, 1977), but the growth retardants such as CCC have the reverse action i.e. they decrease the elongation growth (Humphries *et al.*, 1965; Humphries, 1968; Humphries and Bond, 1969), slow the rate of apical development (Ma and Smith, 1991; Emam and Karimi, 1996), and are expected to act by reducing the amount of active gibberellin available (Bode and Wild, 1984).

Furthermore, Stapper and Fischer (1990) showed that higher density was associated with a significant reduction in number of tillers per plant. The same conclusion has been made by other investigators (Darwinkel, 1978). However, a timely application of CCC, in a crop stand with near-optimum population density has been shown to increase the number of fertile shoots per plant. (Waddington and Cartwright, 1986; Ma and Smith, 1991; Emam and Karimi, 1996).

The rate of apical development has also been shown to be hastened by population density. For example, Kirby and Faris (1970) using a wide range of planting densities (50 to 1600 plants m<sup>2</sup>), have concluded that apical development was hastened by population density. However, Ma and Smith (1991) showed that application of chlormequat at third leaf stage (Zadoks growth stage (ZGS) 13) retarded the apical development of the main shoot in barley. Similar results have

also been reported by others (Waddington and Cartwright, 1986).

Despite detailed studies with growth retardants (such as CCC) in barley showing increased grain number and biomass, little is known about the underlying mechanisms of achieving yield in this way. In the present investigation the short and long term effects of timely application of CCC on barley growth and development at different planting densities were studied.

## MATERIALS AND METHODS

The investigation was designed to compare the responses of a range of plant densities to CCC application at the Agricultural Experiment Station of Shiraz University at Badjgah, 1810 m above sea level, longitude 52°32' E and latitude 29°36' N. Graded seeds (3-3.25 mm) of cultivar "Valfajr" with 99% germination were hand sown with square spacing giving 100, 175, 250, 325 and 400 plants m<sup>2</sup> in forty miniplots of 3x2 m on 8 Nov. 1995.

Regulation of spacing and uniformity of sowing depth was achieved by sowing through perforated plywood sheets with the appropriate density of holes, using a hand dibber to make holes of 5 cm depth. Seedlings which failed to emerge were quickly replaced with matched spares (raised in individual containers) to obtain the exact prescribed density. To all the miniplots, nitrogen and phosphorus were added uniformly (75 kg ha<sup>-1</sup>) at sowing time. The experiment was based upon a randomized complete block design with four replicates. Each replicate consisted of 10 plots of 6 m<sup>2</sup> each. The ten treatments of the experiment were composed of 5 planting densities x 2 plant growth regulator levels (treated and untreated). Plant establishment and survival were excellent in all treatments. No herbicide was applied since plots were regularly hand weeded. During the season all the plots were top-dressed with nitrogen at mid-tillering

and stem elongation, each at the rate of 50 kg N ha<sup>-1</sup>. The CCC treatment was applied as "Arotex Extra" at 1370 g (a.i.) ha<sup>-1</sup> with a precision sprayer at constant pressure of 3 bars at the "lemma primordium stage" of apical development (developmental score DS 3.0 according to the Waddington scale (Waddington *et al*, 1983) which occurred 130 days after sowing. A wetting agent, at 100 ml ha<sup>-1</sup> was added. Rigid screens were used to prevent spray drift. During the growing season frequent small harvests of 5 plants (at 3-day intervals) were taken from pre-designated "sampling stations". Each station was surrounded by at least two guard rows, these frequent small samples continued to be taken up to anthesis (DS 10 according to Waddington scale, judged by anther dehiscence) and also provided some preliminary data for dry matter production and partitioning. True stem length, tiller number per plant, ear length and spikelet number per ear were recorded throughout the growing season. Within each plot an area of 1 m<sup>2</sup> was marked and left for harvesting at crop maturity (ripe grain) which was used for yield component analysis. Dry weights were recorded after the plant material had been oven-dried at 80°C for 72 hours. The data were analyzed by analysis of variance and the means compared by Duncan's new multiple range test (DNMRT).

## RESULTS AND DISCUSSION

The effect of plant density on attributes of growth and development of the main shoot before anthesis are presented in Table 1. The main shoot true stem length was higher in the higher densities, for example, at first harvest, it was 80, 64, 56, 49 and 31 mm in 400, 325, 250, 175 and 100 plants m<sup>-2</sup>, respectively. At the same time the spike initial was also slightly longer at the higher planting densities (6.8, 7.3, 6.7, 5.4 and 5.1 mm in 400, 325, 250, 175 and 100 plants m<sup>-2</sup>,

respectively). These observations are consistent with those reported by Kirby (1967), Willey and Holliday (1971), and Darwinkel (1978) and further confirm the close link between increasing population density and accelerated elongation growth attributed to increased "density induced" GA production (Kirby and Faris, 1970). The longer ear initial and true stem in the higher density were associated with an accelerated rate of apical development (Table 1). This pattern of accelerated development and stem elongation continued during the rapid stem elongation (Zadoks growth stage, ZGS 32 to ZGS 39, see Zadoks *et al* 1974).

In addition, the estimated start of rapid stem elongation phase of the main shoot (ZGS 32, which coincided with DS 4.5) was hastened by 4 to 5 days at higher densities i.e. it occurred at 144 and 149 days after sowing in 400 and 100 plants m<sup>-2</sup>, respectively. However, during the following period of rapid ear growth up to anthesis (from 158 to 172 days after sowing) the difference in the rate of culm elongation was reversed, i.e. it had become slightly in favour of the lower densities and by anthesis the plants of the lower density tended to be slightly taller (the main shoot height was 92.5 and 88.6 cm in 100 and 400 plants m<sup>-2</sup>, respectively).

The lower growth rate at higher densities later in the pre-anthesis, observed in the present investigation, further confirmed the results obtained by other researchers e.g. Kirby and Faris (1972) who attributed the "slow" growth later in the pre-anthesis at supraoptimal plant density to nitrogen deficiency. Also Puckidge and Donald (1967) reported on the rapid rate of leaf senescence in higher plant densities (starting at jointing stage) which led to the "failure" of the foliage, apparently due to the low nitrogen content in the foliage. Kirby (1967) attributed the observed depressed relative growth rate at high densities to high respiration rate per unit area which resulted

Table 1. Effects of plant density on attributes of growth and development of the main shoot before anthesis in winter barley cultivar "Valfajr".

DAS <sup>a</sup>	Plants m <sup>-2</sup>	TS(mm) <sup>b</sup>	Spike length (mm)	ZGS <sup>c</sup>	DS <sup>d</sup>	Spikelet number
144	100	31b <sup>e</sup>	5.1b	31	3.5	60a
	175	49b	5.4b	31	4	64a
	250	56ab	6.7a	31	4	62a
	325	64a	7.3a	32	4.5	59a
	400	80a	6.8a	32	4.5	64a
158	100	222b	65.1b	34	6.5	51a
	175	268b	69.6a	36	7	49a
	250	30 lab	61.6c	37	7.5	44b
	325	331a	58.8c	42	7.5	43b
	400	305a	56. Id	45	8	39c

a Days after sowing. b True stem length. c Zadoks growth stage. d Waddington developmental score (sec Waddington *et*

*ah* 1983). e Means followed by the same letter in each column for each harvest are not significantly different at 5% probability level using DNMRT.

in lower dry matter increments. It could also be attributed to lower photosynthetic rate because of shading.

Spike elongation and development were also affected by population density in much the same way as the extension growth, for example, up to 144 days after sowing. The ear initial of the shoot tended to be longer and at a more advanced stage of apical development in 400 plants m<sup>2</sup> density than in the 100 plants m<sup>2</sup> (Table 1) but the reverse was the case from 158 days after sowing onwards.

At anthesis, the main shoots at higher densities had shorter ears (Fig. 1) and this was due to more extensive "tip death" observed in these shoots. The decrease in spikelet number per ear was observed from 158 days after sowing onwards (Table 1). Abortion of more spikelet primordia during tip death at higher population densities has been previously noted by Emam and Karimi (1996). As expected, the number of shoots per plant also decreased by increasing population density and this trend was carried through until anthesis (Table 2). Indeed, more shoot

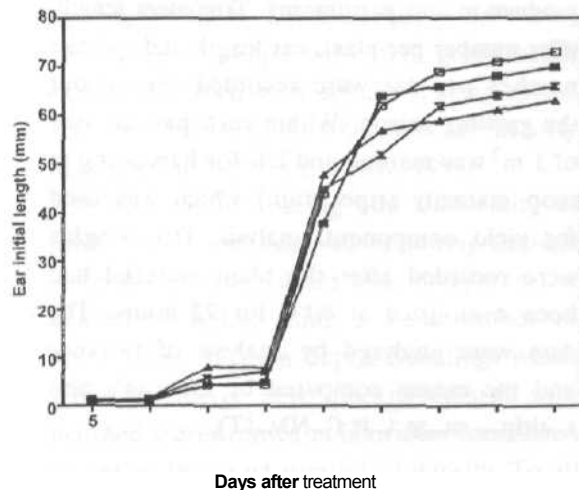


Figure 1. Effect of CCC on main shoot ear length for lowest (100 plants/m<sup>2</sup> n , -a- for control and CCC, respectively) and highest (400 plants/m<sup>2</sup> A , -g. for control and CCC respectively) planting densities up to anthesis.

mortality occurred during stem elongation and ear growth at higher densities than as has been noted by others (Darwinkel, 1978).

The "early" application of CCC, i.e. application at the lemma primordium stage of the most advanced spikelet, affected certain aspects of growth and development in much the

same way as the low population density (100 plants m<sup>2</sup>) in that the rates of both stem elongation and spike development were lower at early stages (Table 3). As reported earlier, the onset of rapid stem elongation was hastened with increased plant density but delayed by the early CCC treatment. This is consistent with the "GA-induced" effect of increasing plant density (Kirby and Faris, 1970) and the "anti GA" mode of action of CCC (Ma and Smith, 1992a). Indeed, such antagonistic effects of high density and the CCC treatment on stem elongation provided indirect evidence in favour of "density induced" and "CCC hindered" GA activity. Thus, it could explain the observation that the effect of CCC treatment on culm elongation resembles that of low density.

Early application of CCC slowed the rate of apical development of the main shoot without any significant effect on its spikelet initiation rate. Similar results are also reported by other researchers (Ma and Smith, 1992b). However, the peak spikelet number in CCC-treated plants were found to be higher (61 and 58 for CCC-treated and control, respectively). Indeed the spike length of the treated plants which was initially shorter than

untreated controls (Table 3) tended to be longer by anthesis (Figure 1). CCC also affected tillering (Table 2), so that treated plants bore more tillers, and this trend was carried through until anthesis. Increase in tiller number per plant following timely application of CCC confirmed the previous findings (Ma and Smith 1992b; Emam and Karimi, 1996).

As for the effect of plant density and CCC on grain yield and its components, it was revealed that: in the absence of CCC, increasing population density (up to 250 plants m<sup>2</sup>) was associated with higher grain yield. However, there was no significant difference in grain yield among higher population densities i.e. from 250 to 400 plants m<sup>2</sup> (Table 4). The lack of any significant difference in grain yield at higher densities (which were in a fairly narrow range) was expected and confirmed the results reported by other authors (Lovelt and Kiby, 1971; Willey and Holliday, 1971). It appeared that the higher planting densities were in a range around the supposed "optimum" density. While higher grain yield was associated with a higher grain number, the latter was the result of higher number of ears m<sup>2</sup>. However, at higher

Table 2. Effect of planting density and CCC on tiller number/plant up to anthesis.

DAS <sup>a</sup>	PGR <sup>b</sup>	(Plants m <sup>-2</sup> )				
		100	175	250	325	400
144	Cont.	8.3 a <sup>c</sup>	5.4 b	5.6 b	3.6 b	3.7 h
	CCC	8.1a	6.1 ab	5.1 ab	4.6 b	4.2 b
158	Cont.	7.4 a	4.4 b	4.1 b	3.2 b	2.7 b
	CCC	7.3 a	5.5 ab	4.2 be	3.7 c	3.6 c
172	Cont.	5.1 a	2.7 b	2.3 be	1.6 c	1.3 c
	CCC	5.6 a	4.6 a	3.3 b	3.4 b	2.2 c

a Days after sowing. b Plant growth regulator. c Means followed by the same letter in each row are not significantly different ;t 5% probability level using DNMR T.

**Table 3.** Short term (14 days after treatment) effects of CCC on attributes of growth and development.

	(Plants m <sup>-2</sup> )				
	100	175	250	325	400
	Pseudo stem length (mm)				
Cont.	125.8	161.2	172.9	195.8	224.7
CCC	109.8*	116.6*	119.2*	126.6*	131.6*
	True stem length (mm)				
Cont.	34.8	65.3	74.5	85.3	119.6
CCC	27.3*	33.5*	38.5*	42.8*	68.7*
	Apex length (mm)				
Cont.	5.3	5.6	7.8	8.1	8.2
CCC	4.7*	5.2 <sup>NS</sup>	5.4*	5.7*	6.6*
	Dry weight (g/plant)				
Cont.	1.6	1.3	1.1	1.1	<b>1.2</b>
CCC	1.4 <sup>NS</sup>	1.2 <sup>NS</sup>	1.1 <sup>NS</sup>	0.8*	<b>0.9</b>

Plant growth regulator.

From the plant base to the ligule of the last fully expanded leaf.

. NS = Significantly different from control at 5% level, and nonsignificant, respectively.

**Table 4.** Effect of population density and CCC on grain yield and its components.

Flams m <sup>-2</sup>	PGR <sup>a</sup>	Grain yield (gm <sup>-2</sup> )	Grain no (m <sup>-2</sup> )	Ear no (ear <sup>-1</sup> )	Grain no (ear <sup>-1</sup> )	Phytomass (gm <sup>-2</sup> )	Harvest index %
100	Cont. <sup>b</sup>	463 b	934 b	280 c	33 a	984 b	47 a
	CCC	510	10270	302	34	1085	47
	%Response	10.1 <sup>NS</sup>	9.8 <sup>NS</sup>	7.8 <sup>NS</sup>	3.3 <sup>NS</sup>	10.3 <sup>NS</sup>	0
175	Con I.	484 ab	10790 ab	348 be	31 ab	1049 b	46 a
	CCC	515	11860	370	32	1197	43
	%Response	6.4 <sup>NS</sup>	9.9 <sup>NS</sup>	6.3 <sup>NS</sup>	3.2 <sup>NS</sup>	14.1*	0
250	Con I.	509 a	11337 a	390 b	29 abc	1204 ab	42 b
	CCC	617	13990	437	32	1402	44
	%Responsc	21.2**	23.4**	12.0 <sup>NS</sup>	10.3 <sup>NS</sup>	16.4*	2.3 <sup>NS</sup>
325	Com.	497 ab	11270 a	424 ab	26 cd	1268 a	39 e
	CCC	530	12270	430	28	1292	41
	%Response	6.6 <sup>NS</sup>	8.9 <sup>NS</sup>	14 <sup>NS</sup>	7.7 <sup>NS</sup>	1.9*	5.1 <sup>NS</sup>
400	Cont.	489 ab	11260 a	469 a	24 b	1292 a	38 c
	CCC	518	12029	523	23	1363	38
	% Response	5.9 <sup>NS</sup> *	6.8 <sup>NS</sup>	11.5*	4.2 <sup>NS</sup>	5.5 <sup>NS</sup>	0

a Plant growth regulator.

b Means followed by the same letter in each column arc not significantly different at 5% probability level using DNMR.

. \*\* . NS = Significantly different from control at 5% , 1% level and nonsignificant, respectively.

densities, i.e. from 250 to 400 plants  $m^{-2}$  increase in ear number  $m^{-2}$  was associated with a slight decrease in ear size, i.e. number of grains/ear, therefore, the highest density led to the lowest grain number per ear (Table 4). Also, increasing plant density (up to 250 plants  $m^{-2}$ ) was associated with increase in phytomass production. However, at higher densities i.e. from 250 to 400 plants  $m^{-2}$  increase in phytomass production was compensated for by reduced harvest index, and therefore, the grain yield was not significantly affected (Table 4). Early CCC application increased the grain yield in all plant densities, but was significant only at 250 plants  $m^{-2}$  level. The increased grain yield was the result of higher grain number  $m^{-2}$  (a greater sink size) which is in agreement with the result reported by other workers (Ma and Smith, 1991; Ma and Smith 1992 a, b). Thus, for overall treatments i.e. five planting densities and two plant growth regulator treatments, grain yield was highly correlated with grain number ( $r=0.98$   $P<0.01$  Fig. 2). Also, the regression of grain yield against phytomass showed that there was a linear positive correlation ( $r=0.70$   $P<0.05$ ) between phytomass and grain yield (Fig. 3). Therefore, it could be suggested that the beneficial effect of CCC achieved through yield components was largely determined before anthesis. Although there was no significant interactive effect between CCC and population density, the present investigation demonstrated that increase in grain yield could be achieved at near "optimum" planting densities i.e. around 250 plants  $m^{-2}$  and by early application of CCC. It was also demonstrated that the "Engledow dilemma" of mutual compensation of yield components faced by breeders could be broken. Since such plant growth regulators as CCC only manipulate the product of gene action (in this case endogenous hormones) it seems theoretically possible that similar grain yield increases could be achieved through

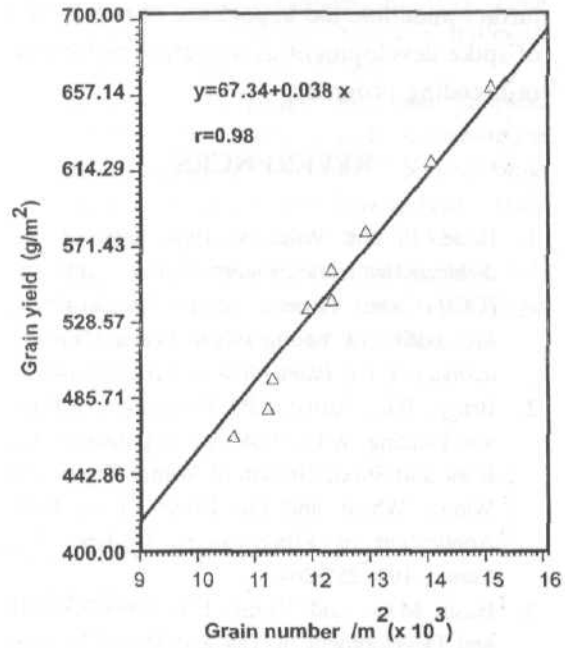


Figure 2. Relationship between the grain yield and grain number upon all treatments (5 densities x 2 PGRs)

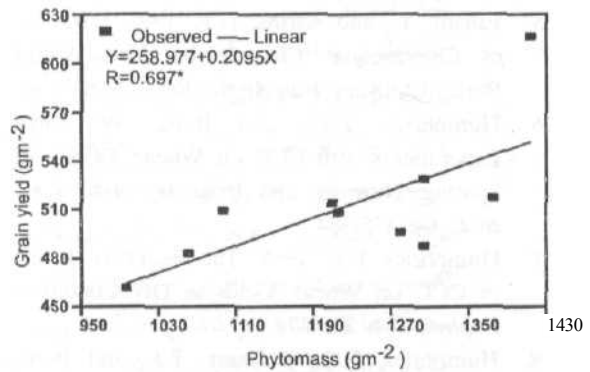


Figure 3. Regression of grain yield against phytomass upon all treatments (5 densities x 2 PGRs)

breeding. Although it might not be easy to select for such criteria as retardation of spike development and culm elongation, selection for such visible characters as shoot uniformity and crown angle, associated with CCC application could offer good possibilities. Our results and those obtained by Bush and Evans (1988) on the effects of *Rht* genes

further underline the importance of monitoring of spike development as a routine procedure in breeding programs.

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## اثر تراکم کاشت و کلرمکوات بر ویژگی‌های مورفولوژیکی و فیزیولوژیکی جو زمستانه رقم والفجر

### چکیده

در یک آزمایش مزرعه‌ای تأثیر پنج تراکم کاشت (۱۰۰، ۱۷۵، ۲۵۰، ۳۲۵ و ۴۰۰ بوته در مترمربع) با و بدون کاربرد کلرمکوات کلرید (۲ - کلرواتیل ۳ - متیل آمونیوم کلرید، CCC) بر رشد، نمو و عملکرد دانه جو زمستانه رقم والفجر مورد مطالعه قرار گرفت. نتایج به دست آمده نشان داد که افزایش تراکم گیاهی موجب تسریع آهنگ نمو و رشد طولی ساقه تا زمان شروع رشد طولی ساقه (مرحله رشد زیداکس ۳۲) گردید. لیکن، در طول دوره رشد طولی ساقه و رشد سنبله این روند معکوس گردید و سرعت نمو و رشد طولی ساقه در تراکم‌های کمتر شدیدتر بود. افزایش تراکم تا ۲۵۰ بوته در مترمربع عملکرد دانه را افزایش داد، لیکن، از ۲۵۰ تا ۴۰۰ بوته در مترمربع تغییر معنی داری در عملکرد دانه ایجاد نکرد. به نظر می‌رسد تراکم‌های ۲۵۰ تا ۴۰۰ بوته در مترمربع در محدوده تراکم "بهینه" بوده است. کاربرد CCC بر روی شاخ و برگ بوته‌ها آهنگ نمو و رشد طولی ساقه را در همه تراکم‌ها کاهش داد اما، تأثیری بر سرعت آغازش سنبلک‌ها نداشت. این کاهش آهنگ نمو در بوته‌های تیمار شده با CCC در ابتدا با کاهش تجمع ماده خشک همراه بود لیکن، پیش از گلدهی این روند معکوس گردید، بنحوی که وزن خشک نسبت به شاهد در هنگام گلدهی زیادتر بود و نهایتاً عملکرد دانه آنها هم بیشتر بود. شناخت دقیقتر تأثیر متقابل CCC و تراکم بوته نیاز به پژوهش‌های بیشتری دارد.