



Effect of explant length and density on *in vitro* shoot formation and growth of Pineapple (*Ananas comosus* L. Merr.) cv Moris

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Received: 9 February 2017. / Accepted: 26 October 2017

Doi: <https://doi.org/10.54172/mjsc.v32i2.181>

Abstract: The effect of explants length (5, 10, 15, 20, 25 mm long shoots) and density (one, two, three, four, five shoots per culture) on the *in vitro* shoot formation of Moris pineapple were assessed using average shoot formation per explant and total shoots and the frequency of shoots of different shoot length (≤ 5 ; 6-10; 11-15; 16-20; 21-25 and ≥ 26 mm long) per one liter of medium. Of all combinations of explants length and density, using of 25 mm long shoots at a density of one per culture resulted in highest shoot formation (8.3 shoots), and at density of three and four resulted in longest shoots (21.7 mm). The highest total shoots per one liter of medium (2800 shoots) obtained when 15 mm long explants were used at a density of four explants per culture. Using of 10 mm long shoots at a density of two per culture resulted in lowest shoot formation (2.7 shoots) and shortest shoots (5 mm long) per explant and lowest total shoots (233 shoots) per liter of medium. Overall explants density, the percentage of shoots of different length ≤ 5 ; 6-10; 11-15; 16-20; 21-25 and ≥ 26 mm-long per liter of medium were 22.4; 21.9; 22.3; 16.9; 6.6 and 9.3% respectively. To obtain highest shoot formation per explant or highest total shoots per liter of medium, the according to the length of available shoots.

Key words: Explant density; Explant length; Total shoots; *Ananas comosus*.

INTRODUCTION

In vitro multiplication of pineapple are usually done by repeating cultures of the multiple shoots complex as whole (Almeida *et al.*, 2002), segmenting the multiple shoots into clusters of 2 to 5 shoots (Escalona *et al.*, 1999, Firoozabady and Gutterson 2003, Firoozabady and Moy 2004, Hamad and Taha 2009), and separating the multiple into individual shoot and subculture as intact (Mathews and Rangan 1979, Fernando 1986, Soneji *et al.*, 2002, Sriparaya *et al.*, 2003, Khan, *et al.*, 2004, Hamad and Taha 2008, Dutta *et al.*, 2013, Akin-Idowu *et al.*, 2014, Nelson *et al.*, 2015), or as halved shoot (Almeida *et al.*, 2002, Bhatia and Ashwath 2002) into fresh medium. Removing of the shoot leaves and

culturing of stem core (Firoozabady and Gutterson 2003), sectioning and chopping of shoot base into granular (Teng 1997) were also suggested. Culturing of intact separated shoots were done at density of one (Fernando 1986, ; Devi, *et al.*, 1997, Dal Vesco *et al.*, 2001, Akbar *et al.*, 2003, Sriparaya *et al.*, 2003, Hamad and Taha 2008, Dutta *et al.*, 2013, Nelson *et al.*, 2015), two (Soneji *et al.*, 2002, Be and Debergh 2006), three (Hamad and Taha 2003), four (Khan, *et al.*, 2004, Hamad and Taha 2009), five (Daquinta *et al.*, 1997, Escalona *et al.*, 2003, Pérez *et al.*, 2009) eight (Dal Vesco *et al.*, 2001), and ten shoots (Zuraida *et al.*, 2011) per culture. Clusters of 2 to 3 shoots were cultured at density of four (Hamad and Taha, 2009) and five clusters (Escalona *et al.*, 1999) in a conventional system,

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and at density of 150 and 200 clusters (Firoozabady and Gutterson 2003) in a temporarily immersion system. The size of explants used for multiplication ranged from 1 mm chopped granular and 3 mm shoots base section (Teng, 1997) to 3x3 mm stem core and 5 to 20 mm long shoots (Firoozabady and Gutterson 2003), 15 to 35 mm (Dal Vesco *et al.*, 2001), 20 to 30 mm (Escalona *et al.*, 1999) and 50 to 100 mm long shoots (Bordoloi and Sarma, 1993; Teng 1997).

Average shoot formation and shoot length per explant are the most commonly used parameters for assessment of *in vitro* multiplication treatments. Treatments with the highest average of shoot formation were considered the best and suggested for large-scale production of *in vitro* shoots. However, an equal average of shoots formation and shoot length does not mean all of the *in vitro* obtained shoots are of equal length. Dal Vesco *et al.*, (2001) reported that the shoot formation rate and frequency of shoots of different length varied among explants of different size. Escalona *et al.*, (1999) found that the total shoots production and frequencies of shoots of different lengths depended on incubation period and explants density. Therefore, it is expected that if all of the shoots produced in a cycle of multiplication used for next cycle at a constant explant density, treatments with equal average of shoot formation could produce different total of shoots as a result of different frequency of shoot length. Knowing the effect of combinations of explants length and density on shoot formation and frequency of shoots of different length would help in deciding how the following cycle of multiplication should be managed. Different explants density might suggest for explants of different shoot lengths to obtain the optimal shoot formation from each shoot length. The objective of this study is to compare the effect of five different explants length (5, 10, 15, 20 and 25 mm long) cultured at five different density (one, two, three, four and five) on Moris pineapple shoot formation per explant and total shoots and frequency of shoots of different length per one liter of medium.

MATERIALS AND METHODS

Full strength liquid MS medium (Murashige and Skoog 1962) enriched with sucrose at 20 g/l and 6-benzylaminopurine (BAP) at 2.0 mg/l and adjusted to pH 5.0 was prepared and autoclaved for 20 minutes at 121⁰ C and 1.5 kg/ cm². The medium was dispensed under laminar cabinet floor into 75 culture tubes (10 ml per tube) using a sterilized syringe. Multiple shoots complex were picked under laminar cabinet from Moris stock cultures, placed on sterilized petri dish and separated into individual shoots. Shoots were arranged in groups of different length (5, 10, 15, 20 and 25 mm long) and shoots from each group were cultured at densities of one, two, three, four and five shoots per culture. Three culture tubes were used for each combination of explant length and density. After two months of incubation under a constant temperature of 25⁰ C and 16 hours of light, the multiple shoots complexes were picked out from each culture tube and separated into individual shoots for counting and measuring of shoot length. Each culture tube of the same combination of explants density and length was considered as a replicate and the recorded data were rearranged into 7 different tables. One table was for total shoots per culture (irrespective of the shoot length). Then the total shoots per each culture tube were assorted into 6 different shoot lengths ranks (< 5; 6-10; 11-15; 16-20; 21-25; > 26 mm) and 6 tables were constructed for the number of shoots per each shoot length rank. To avoid the zero values for shoot number of some of the shoot length ranks, the data were transformed by adding 0.01 to the original data of all of the 6 tables. The table of total shoots (irrespective of shoot length) and tables of total shoots of each shoot length rank per culture were converted to tables of shoot formation per explant by dividing by the explants density per culture, and to tables of total shoots per one liter of medium by multiplying by 100. The average shoot length data were established by dividing the sum of the length of all shoots per culture by the total shoots per culture and converted to average shoot length per explant by dividing by the explant density per culture. The

data of each parameter were analyzed by two ways ANOVA and the treatments means separated by Duncan Multiple Range Test at $p \leq 0.05$ using SPSS statistical package No.11.

RESULTS

Analysis of variance indicated that the explants length induced significant independent direct ($p \leq 0.00001$) and indirect ($p \leq 0.0015$) effect via interaction with explant density on shoot formation, and also significant independent direct ($p \leq 0.00001$) and indirect ($p \leq 0.00001$) effect on shoot length. That is both growth parameters (shoot formation and length) were under direct and indirect effect of the explants length. The explants density, on the other hand, also induced significant direct ($p \leq 0.0001$) and indirect ($p \leq 0.0015$) effect on shoot length, but its effect on shoot formation was only indirect via interaction with explants length ($p \leq 0.0016$). The direct effect of explant density on the shoot formation was insignificant ($p \leq 0.3356$). Overall explants density, the explants could be divided into two groups, shorter explants (5 and 10 mm long) resulted in statically equal and lowest (3.5 and 4.3 shoots) and longer explants (15, 20 and 25 mm long) resulted in statically equal and highest (5.5, 6.1 and 5.5 shoots) shoot formation per explants. The shoot length increased from 10.7 to 18.6 mm as the explants length increased from 5 to 25 mm (Table, 1). In addition, overall explants density, the total shoots per liter of medium increased from 1113 when the explants were 5 mm long to 1680 and 1747 shoots when the explants were 15 and 20 mm long respectively but declined to 1520 shoots when 25 mm long explants were used (Table, 1). On the other hand, overall explants length, the shoot length increased as the explants density increased up to three explants per culture. Using of one explant per culture resulted in shortest shoots (9.9 mm) and the shoot length increased to 15.1 mm long as the explants density increased to three explants per culture and remained stable (16.1 and 15.7 mm) at densities of four and five explants per culture. Overall explants length, different explants densities resulted in equal shoot

formation per explant (about 5 shoots) irrespective of the explants density per culture. However, increasing the explants density increased the total shoots per liter of medium from 500 at density of one to 2160 and 2200 shoots at densities of four and five explants per culture (Table, 1).

Of all combinations, using of 5 and 10 mm long explants at a density of one explant resulted in the lowest (2 and 3 shoots per explants) and using of 25 mm long explants also at a density of one explant resulted in the highest shoot formation (8.3 shoots per explants). However, the highest total shoots per liter of medium (2800 shoots) obtained using 15 mm long explants at a density of four explants and the lowest total (233 shoots) obtained using 10 mm long explants at a density of one explant per culture (Table, 1). The highest shoot formation from using 5, 10, 15, 20 and 25 mm long explants were 3.7; 5.7; 7.0; 6.7 and 8.3 shoots obtained when these explants were used at density of three, two, four, four and one explants per culture, and the lowest were 2.0; 3.7; 4.3; 4.7 and 3.7 shoots obtained when the explants were used at density of one, five, five, five and three explants per culture respectively. The highest total shoots per liter of medium from the 5 mm long (2000 shoots), 10 mm long (1850 shoots) and 25 mm long (2667 shoots) explants all were obtained when the explants were cultured at density of five explants per culture while the highest total shoots from the 15 (2800 shoots) and 20 mm long (2667 shoots) explants were obtained when the explants were cultured at density of four explants per culture. However, the lowest total shoots produced from the 5, 10, 15, 20 and 25 mm long explants were 267, 233, 500, 667 and 833 shoots all obtained when explants were cultured at a density of one explant per culture. Similar to its effect on shoot formation rate and total shoot, the effect of explants density on the shoots length varied depending on the explants length. When the 15 and 25 mm long explants were used, the length of the *in vitro* obtained shoot increased respectively from 12 and 14 mm to 16 and 22 mm as the explants density increased from one to four explants and then decreased to

13 and 19 mm at a density of five. In case of the 5, 10 and 20 mm long explants, the length of the *in vitro* obtained shoot was generally increased as

the density increased up to five explants per culture (Table, 1).

Table. (1). Effect of explants length and density on Moris pineapple *in vitro* shoot formation and length per explant and total shoots per liter of medium.

Explants length (mm)	Explants density					Average
	1	2	3	4	5	
Shoots per explants						
5	2.7 _{gh}	3.0 _{fgh}	3.7 _{efgh}	4.0 _{defgh}	4.0 _{defgh}	3.5 _B
10	2.3 _h	5.7 _{bcde}	5.3 _{bcdef}	4.7 _{bcdefgh}	3.7 _{efgh}	4.3 _B
15	5.0 _{bcdefg}	4.7 _{bcdefgh}	6.7 _{abc}	7.0 _{ab}	4.3 _{bcdefgh}	5.5 _A
20	6.7 _{abc}	6.3 _{abcd}	6.0 _{abcde}	6.7 _{abc}	4.7 _{bcdefgh}	6.1 _A
25	8.3 _a	5.7 _{bcde}	3.7 _{efgh}	4.7 _{bcdefgh}	5.3 _{bcdef}	5.5 _A
Average	5.0 _{NS}	5.1 _{NS}	5.1 _{NS}	5.4 _{NS}	4.4 _{NS}	
Shoot length (mm)						
5	6.3 _{gh}	10.7 _{fg}	14.3 _{cdef}	9.7 _{fg}	12.3 _{ef}	10.7 _D
10	5.0 _h	12.3 _{ef}	12.3 _{ef}	15.0 _{bcdef}	14.7 _{bcdef}	11.7 _{CD}
15	12.0 _{ef}	11.7 _{ef}	14.7 _{bcdef}	16.3 _{bcde}	13.3 _{def}	13.6 _{BC}
20	13.0 _{def}	12.3 _{ef}	12.7 _{def}	17.7 _{abcd}	19.7 _{ab}	15.1 _B
25	14.3 _{cdef}	16.7 _{bcde}	21.7 _a	21.7 _a	18.7 _{abc}	18.6 _A
Average	9.9 _C	12.7 _B	15.1 _A	16.1 _A	15.7 _A	
Total shoot per liter						
5	266.7 _{jk}	600.0 _{hijk}	1100 _{ghi}	1600 _{defg}	2000 _{bcd}	1113.3 _C
10	233.3 _k	1133.3 _{fghi}	1600 _{defg}	1866.7 _{cde}	1833.3 _{cde}	1333.3 _{BC}
15	500.0 _{ijk}	933.3 _{ghij}	2000 _{bcd}	2800 _a	2166.7 _{abcd}	1680 _A
20	666.7 _{hijk}	1266.7 _{efgh}	1800 _{cdef}	2666.7 _{ab}	2333.3 _{abc}	1746.7 _A
25	833.3 _{hijk}	1133.3 _{fghi}	1100 _{ghi}	1866.7 _{cde}	2666.7 _{ab}	1520 _{AB}
Average	500.0 _D	1013.3 _C	1520 _B	2160 _A	2200 _A	

Means of each parameter followed by differet letter were significantly different at $p \leq 0.05$ according to Duncun Multiple Range Test Total shoots per liter of medium computed by multiplying the average of total shoots of each combination of explants length and explants density by 100. (Shoots per explant x explants density x 100).

The effect of the explants density and length were not limited only to the average shoots formation per explant and total shoots per liter of medium, but also extended to the frequency of shoots of different length per liter of medium (Table, 2). Different explant densities which resulted in equal total shoots per liter resulted in different frequency of shoots of different length. For instance, density of four and five explants per culture resulted in statistically equal total of 2160 and 2200 shoots per liter respectively (Table, 1). In both explants density per culture (Table, 2), the total shoots which were within the shoot length range of 6-10 mm (400 and 533 shoots), shoot length range of 11-15 mm (480 and 467 shoots) and shoot length range of ≥ 26 mm long (267 and

233 shoots) were also statistically equal. However, while the total shoots which were within shoot length range of < 5 mm (467 shoots) and shoot length range of 16-20 mm (367 shoots) obtained from density of five explants per culture were significantly higher than the 400 and 347 shoots obtained at density of four explants per culture, the total shoots per liter within the shoot length range of 21- 25 mm long obtained at density of five (133 shoots) were significantly less than that (267 shoots) produced at density of four explants per culture (Table, 2). Using of one, two and three explants per culture resulted in significant different total of 500, 1013 and 1560 shoots per liter and also in significant different total of shoots that were shorter than 5 mm (160,

240 and 300 shoots), shoots that were within 6-10 mm long (107, 213 and 400 shoots), shoots that were within 11- 15 mm (93, 227 and 380 shoots) and shoots that were within 16- 20 mm long (67, 160 and 240 shoots). However, the different in total shoots per liter that were within 21- 25 mm long (40, 107 and 80 shoots) and were longer than 26 mm (33, 67 and 120 shoots) was not significant.

The frequency of shoots of different length per one liter of medium was also affected by length of the explants (Table, 2). The frequency of shoots which shorter than 6 mm and those within 6-10 mm long among the total shoots per liter of medium were not effected by the explants length (statistically equal). At each explant length (5, 10, 15, 20 and 25 mm long), about 313 and 331 shoots of the total shoots per liter were within the shoot length range < 5 and 6- 10 mm long respectively (Table, 2). However, the total shoots within the shoot length range of 11- 15 mm; 16-20 mm; 21 -25 mm and > 26 mm long were significantly affected by the explants length. When 5, 10 and 15 mm long explants were used, the total of shoots longer than 26 mm were 73, 40 and 113 shoots while when 20 and 25 mm long explants were used the total of shoots longer than 26 mm were 227 and 267 shoots respectively. Generally, the majority of shoots (66 %) were within the range of 5 to 15 mm long and few within range 16 to 30 mm long. Overall, the percentage of the ≤ 5 ; 6-10; 11-15; 16-20; 21-25

and ≥ 26 mm long shoots were 21; 22; 22; 16; 8 and 10 % of the total shoots expected per one liter of medium respectively.

DISCUSSION

This study demonstrated that for each explants length there were different optimal explants density for shoot formation per explant and total shoots per liter of medium and vice versa. Explants of different lengths had different range (highest and lowest) of shoot formation per explant that depended on the explants length and different range (highest and lowest) of total shoots per liter of medium that depended on the explants density (Table, 2). In addition, combinations of explant lengths and densities that may result in equal total shoots per liter could result in different frequency of shoots of different length. Generally, any cycle of multiplication would result in shoots of different lengths. For physiological study, few selected shoots usually 15 to 20 mm long are used for investigation of factors effecting the shoot formation and elongation. This study supported the selective use of 15 to 20 mm long explants as the most competent shoot for experimental purposes particularly at density of four explants per culture. The different between explants density was insignificant and overall explants density the 15-20 mm long explants resulted in highest average of shoot formation per explant (Table, 2).

Table (2). Effect of explants density and explants length on the frequency of shoots of different length per total shoots per liter of medium.

Factors	Shoot length range (mm) / liter						Average
	< 5	(6-10)	(11- 15)	(16- 20)	(21- 25)	> 26	
Explant density	Average overall explants length						
1	160d	107b	93c	67d	40b	33b	500D
2	240cd	213b	227b	160cd	107b	67b	1013.3C
3	300bc	400a	380a	240bc	80b	120b	1520B
4	400ab	400a	480a	347ab	267a	267a	2160A
5	467a	533a	467a	367a	133b	233a	2200A
Average	313	331	329	236	125	144	
%	21.16	22.38	22.24	15.96	8.45	9.74	100%
Explant length(mm)	Average overall explants density						
5	273ns	293ns	273ab	200b	0b	73b	1113.3C
10	353ns	293ns	247b	220ab	180a	40b	1333.3BC
15	347ns	340ns	387a	333a	160a	113b	1680A
20	347ns	407ns	373a	240ab	153a	227a	1746.7A
25	247ns	320ns	367a	187b	133a	267a	1520AB
Average	313	331	329	236	125	144	
%	21.16	22.38	22.24	15.96	8.45	9.74	100%

Means of each column followed by different letter were significantly different at $p \leq 0.05$ according to Duncun Multiple Range Test (ns. not significant) Total shoots and frequency of shoots of different length per liter were computed as presented in Material and Methods section

However, for commercial micropropagation all of the shoot produced at any cycle of multiplication should be reused for the next cycle. Use of fixed explants density for all explants irrespective of the explants length simplify the procedure but at expense of optimum shoot formation per explant and optimum total shoots per liter of medium. Improper explants density could cause great loss on shoot formation ability of the explants particularly if the explants length was longer than 25 mm. Subtraction of highest from the lowest shoot formation obtained from each explant length (Table, 2) revealed that if the explants length were 5, 10, 15, 20 and 25 mm long, using of improper explants density could cause loss of possibly obtainable 1.7; 2.0; 2.7; 2.0 and 4.6 shoots per explant respectively. The effect of presence of more than one explants in one culture if the explants length was 25 mm long is more prominent than other explant lengths. It caused loss of about five shoots while the other caused loss of two shoots. That is using of improper combination of explants length and density means losing of 30 to 55 % of the shoot formation capacity even before the multiplication started.

Similar, subtraction of highest total shoots per liter of medium obtained from each explant length from its lowest total shoots showed that if the explants length were 5, 10, 15, 20 and 25 mm long using of improper explants density could cause loss of 1733;1600; 1834; 2300 and 2000 of possibly obtainable total shoots per one liter of medium, Improper density caused the greatest loss (2300 shoots) of possibly obtainable total shoots per liter of medium if 20 mm long explants were used for multiplication. Hence, for highest shoot formation rate and total shoots per liter each shoot should be cultured at different density according to its length. That is for highest shoot formation per explant, the 25 mm long explants should be cultured individually, the 10 mm long at density of two, the 15 and 20 mm long at density of four and the 5 mm long at density of three explants per culture. For highest total shoot per liter of medium the 5, 10 and 25 mm long explants should be used at density of five and the 15 and 20 mm long explants at density of four.

The difference in shoot formation and elongation and total shoots per liter of medium at different combination of explants density and length could

be attributed to competition, number of axillary buds per explant and changes on physical and chemical environment within the culture tube. The shoot formation and elongation of the shoots depended mainly on the explant length and explants of different length responded differently to presence of other explants in the same culture. At equal explants density, longer explants produced more shoots than shorter ones. Longer explants have more axillary buds than shorter ones and would form more shoots than shorter explants. The shoot formation of shorter explants (5, 10 and 15 mm long) increased as the density increased up to 3 explants per culture while the shoot formation of longer explants (20 and 25 mm long) decreased (Table, 1). Longer explants used more nutrients than shorter ones and the amount of nutrient used by five of 5 mm long explants might be less than that used by two of 25 mm long explants. The nutrient content of 10 ml of MS medium could be more than that required for three short explants but less than that required for two long explants. Stability, gradual and sharp decline of shoot formation rate in case of 5, 10 and 15 mm long explants when the explants density was more than three explants per culture supported the nutrient competition. However, formation of more shoots from the 25 mm long explants cultured at density of 5 explants than at density of 4 and 3 explants suggested that in culture having more than three explants, other factors besides competition for nutrient was involved in shoot formation. Konan *et al.*, (2007) reported that at density of 3 shoots per vessel, the rooting of oil palm shoots was affected by coupling factor related to differences in the shoots size. That is rooting of one shoot affected the rooting of the others and mixing longer shoots with shorter ones improved the rooting of all shoots within the culture. In this study, mixing of explants of different lengths (sizes) per single culture tube was not tested. However, the difference in shoot formation rate among the explants of different length and their different responses to explants density per culture indicated that the shoot formations of Moris pineapple were under coupling effect.

CONCLUSIONS

In conclusion, optimization of *in vitro* multiplication system requires selection of the best explants density for the shoots of different length. In addition, besides comparing averages of shoot formation and shoot length per explant, the total shoots per liter of medium and the frequency of shoots of different length should also be considered for assessment of different multiplication treatments. Estimation of total shoots per liter and frequency of shoots of different length is essential for better management and planning of the next multiplication cycle. The shoots should be assorted according to its length and cultured at the proper density according to physiologist and propagator goals. The frequency of shoot within the length range ≤ 5 , 6-10, 11- 15, 16- 20, 21- 25, ≥ 25 was 21; 22; 22; 16; 8 and 10 % and each length should be cultured at proper density. For higher rate 5, 10 and 25 mm long explants should be cultured at density of three, two and one shoot per culture respectively while for highest total shoots per liter of medium all should be cultured at density of five shoots per culture. In case of 15 and 20 mm long shoots, density of four shoots per culture resulted in highest rate and highest total shoots per liter of medium.

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تأثير طول وكثافة العزلة على تكوين ونمو الفريعات في مزرعة أناس صنف موريس

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تاريخ الاستلام: 9 فبراير 2017 / تاريخ القبول: 26 أكتوبر 2017.

<https://doi.org/10.54172/mjisc.v32i2.181>:Doi

المستخلص: تم تقييم تأثير طول العزلة (5، 10، 15، 20 و 25 ملم) وكثافة العزلة (عزلة واحدة، عزلتان، ثلاث، أربع وخمس عزلات) على تكوين ونمو الفريعات الممكن الحصول عليها من عزلة أناس صنف موريس باستخدام متوسط عدد الفريعات من العزلة الواحدة والعدد الإجمالي للفريعات وكذلك نسبة الفريعات الأقل من 5، بين 6-10، 11-15، 16-20، 21-25 وأطول من 26 ملم الممكن الحصول عليها فيما لو استخدم لتر واحد من الوسط. من كل التوليفات من طول وكثافة العزلة تبين أن استخدام عزلة بطول 25 ملم وكثافة عزلة واحدة في المزرعة الواحدة أدى إلى الحصول على أعلى متوسط من الفريعات (8.3 نمو) وعند زراعتها بكثافة ثلاث وأربع عزلات أدت إلى الحصول على أكبر طول للفريع الواحد (21.7 ملم) بينما أعلى إجمالي عدد من الفريعات الممكن الحصول عليها فيما لو استخدم لتر من الوسط (2800 نمو) تم الحصول عليه عند استخدام عزلة بطول 15 ملم بكثافة ثلاث عزلات في المزرعة الواحدة. استخدام عزلة بطول 10 ملم وكثافة عزلتين في المزرعة أعطى أقل متوسط من الفريعات المتكونة من العزلة الواحدة (2.7 نمو) وأقصر طول (5 ملم) وأقل إجمالي عدد من الفريعات (233 نمو) في اللتر الواحد من الوسط. المتوسط العام لكل كثافات العزلة أظهر أن نسبة الفريعات ذات الأطوال أقل من 5: بين 6-11:10-15:16-20:21-25 وأطول من 26 ملم من إجمالي عدد الفريعات الممكن الحصول عليها من استخدام لتر واحد من الوسط كانت 22.4 : 21.9 : 22.3 : 16.9 : 6.6 و 9.3 على التوالي .

الكلمات المفتاحية: كثافة العزلة، طول العزلة، إجمالي عدد الفريعات، الأناس.