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Growth and minituber yield response of potato plantlets in micropropagation to different plant spacing under greenhouse conditions

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Abstract

The present research work was conducted to find out the most suitable inter and intra spacing for Greenhouse transplantation of *in vitro* grown potato plantlets in peat moss soil in order to get maximum seed potato yield. There were nine different combinations of inter and intra row spacing (30x15, 30x10, 10x15, 20x5, 30x5, 10x5, 20x15, 10x10, 20x10cm) in completely randomized design. Regarding vegetative growth, maximum plant height i.e 59.01 cm, 58.92 cm, 57.01 cm was observed at 30cmx5cm, 20cmx5cm, and 10cmx5cm, respectively. Greater number of minitubers in each tuber category i.e. large, medium and small were obtained at greater inter and intra plant spacing , whereas the maximum average tubers yield per plant (165.0g) were recorded at spacing of 30cm x15cm followed by the spacing 20cm x10cm where the average yield per plant was 145.38g. It was concluded that the plant spacing of 30x15cm is the best for potato plantlets transplantation in the greenhouse benches in order to get maximum minituber yield.

Keywords: Inter and intra plant spacing, Potato, Growth parameters, Minituber yield

Introduction

Vegetables are rich and comparatively cheaper source of vitamins. Potato (*Solanum tuberosum*) is one of them. It is a starchy, tuberous crop from the perennial night shade family *Solanaceae*. Potato plays an important role to mitigate the food and nutritional requirements of the fast growing population of developing countries of the world. The balance of protein to calories and the composition of minerals make potato second only to eggs in nutritional value as a single source (Swaminatha and Sawyer, 1983).

Among the root and tuber crops, it ranks first followed by cassava, sweet potatoes and yams (Hawkes, 1990; FAO, 2008). There are a number of constraints that negatively affect potato production in the world and these include lack of quality seed potato, declining soil fertility and structure due to poor management practice, diseases etc (Demo *et al*., 2007, 2009).

In Pakistan, it is also an important vegetable with an area and production of 177.3 thousands hectares and 3977.6 thousand tons, respectively during 2015-16 (GOP 2016). Although potato production in Pakistan has increased many folds but it's per acre yield is far less than in other parts of the world (Malik, 1995).

Among the various factors responsible for low per acre potato production, potato diseases are considered to be the most important. More than 18 potato diseases are reported in Pakistan, of which 13 are of common occurrence. Their importance, however, varies considerably in different potato growing areas (Ahmad *et al.,* 1991).

Tissue culture helps in the production of disease free potato because shortage of good quality seed has been recognized as the single most important factor limiting potato production in the developing countries. Production of quality planting material is essential not only for improving domestic potato productivity but also to ensure commercial quality as required under

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international agreements.

Plant tissue culture is widely used to produce clones of a plant through micro propagation. In vitro micro propagation is an alternative to conventional propagation of potatoes (Chandra and Birhman, 1994). In vitro propagation methods using meristem tips, nodal cuttings and micro tubers are more reliable for maintaining genetic integrity of the multiplied clones. In vitro produced disease free potato clones combined with conventional multiplication methods have become an integral part of seed production in many countries (Naik and Sarkar, 2000). Mini tubers can be produced throughout the year and are principally used for the production of pre-basic or basic seed by direct field planting (Lommen, 1999, Ritter *et al*., 2001).

Appropriate plant density is a key for gainful production of potato in various environments. The possibility of securing high yield depends much upon maintenance of optimum number of plants per unit area and their spatial arrangement in the field. Many studies have been conducted to establish the optimal combination of planting distance for a certain environment not only for potato but many other plants as well (Zerga *et al* 2017,Idoko *et al* 2017, Sultana and Shahiduzzaman 2016, Mahmood *et al,* 2015) etc.

It is well known that plant density (inter and intra-row spacing) is very important aspect of potato production since it significantly affects number of tubers per plant, tuber weight, tuber yield and size (Arega et al, 2018). According to Hani et al., (2018) increase in plant density decreases the mean tuber size due to increased inter plant competition for light, water and nutrients. However, the optimal planting density differs depending on the environmental conditions and cultivars. According to Gulluoglu and Arioglu (2009) closer spacing reduced tuber number per hill, average tuber weight, tuber yield per hill.

Since, quality of potato seed is a key factor in profitable potato production, it is imperative to identify appropriate spacing for seed potato productions. Previous studies were conducted in the field or uncontrolled environment and no work has been done so far on adequate plant spacing for tissue cultured plantlets transplantation in green house to obtain maximum mini-tubers production. The present study was conducted to optimize a protocol for best inter and intra row spacing for tissue cultured potato plantlets transplantation on greenhouse tables in order to get maximum mini tubers yield and size.

Materials and Methods

The present research work was performed at tissue culture laboratory and greenhouse, Hazara Agriculture Research Station, Abbottabad in 2018. Five liters of MS media (Murashige and Skoog media 1962) was prepared from the stock solutions (Table 1) of macro and micro salts with 1 mgl^{-1}Ca-pentothenate, 100mgl^{-1} Myoinositol and 30gl^{-1} sucrose. The pH was adjusted to 5.8 with either 0.1N HCl or 0.1N NaOH. The media was poured in to the test tubes @ 10ml per test tube and were autoclaved at 121°C at 15 psi for 15 minutes.

About 30 days old disease free plants of potato cultivar Desiree were selected from the Tissue culture laboratory as explants (fig. 1). The explants were cut in to nodal segments and inoculated into test tubes. These cultured test tubes were incubated into growth chamber for 30 days at 18°C under photoperiod of 16h light and 8 hours dark (Fig. 1).

After 30 days the plantlets were transplanted in peat moss soil on greenhouse tables. There were nine different combinations of inter and intra row spacing. Plants of S1 having distance of row-row 30cm x plant-plant 15cm, S2(30cm x10cm), S3 (10cm x15cm), S4 (20cm x5cm) , S5(30cm x5cm) , S6(10cm x5cm), S7(20cm x15cm) , S8(10cm x10cm) and S9 having distance of (20cm x10cm) respectively.

The data on growth parameters were recorded for the each spacing after every 20 days. The study parameters were shoot length, number of leaves, inter nodal distance, number of nodes, number of branches, number of leaves per branch, tuber weight, tuber diameter, number of eyes and average tuber yield per plant.

The experiment was conducted using completely randomized design (CRD). The data were analyzed by Analysis of Variance (ANOVA) and Least Significance Difference test was used for significance of results at 95% level of confidence. All the statistical analysis were done using computer software Statistix 8.1.

Figure 1. Plantlets of potato variety Desiree are ready to be transplanted in peat moss on the greenhouse tables.

Results

Each treatment showed different results regarding vegetative growth parameters at different spacing.

Shoot length

The data regarding shoot length in all nine inter and intra row spacing combinations were differed significantly (P≤0.05) after 10 days of transplantation (Table 1). The plants of spacing treatments 20 x 5 cm and 30 x 5 cm showed higher shoot length (9.26 cm) and (9.27 cm), respectively, whereas the spacing 20 x 10 cm showed significantly minimum shoot length i.e 6.77 cm (Table 1).

Table 1. Effect of different inter (r-r) and intra (p-p) plant spacing on various growth parameters of potato plantlets Desiree after 10 days of transplantation in peat moss on Green house tables.

Plant spacing (r-r x p-p)* (cm)	Shoot length (cm)	No of leaves	No of nodes
S1(30 x 15)	8.27 abc	12.37 ab	10.0 ab
S2 (30 x 10)	7.26 cd	11.87 ab	9.37 ab
S3(10 x 15)	8.87 ab	14.12 a	10.2 ab
S4(20 x 5)	9.26 a	13.25 ab	11.0 a
S5(30 x 5)	9.27 a	13.12 ab	10.3 ab
S6(10 x 5)	7.63 bcd	11.37 b	8.87 b
S7(20 x 15)	7.00 cd	12.00 ab	9.25ab
S8(10 x 10)	7.26 cd	12.50 ab	9.87ab
S9(20 x 10)	6.77 d	12.00 ab	9.37ab

Different alphabets with each mean value in a column indicates significant differences of mean value at P≤0.05. *(r-r = row to row; p-p = plant to plant distance).

After 30 days of transplantation a significant (P≤0.05) increase in shoot length was found at spacing 20x5cm; 10x15 cm and 30x5cm (Table 2). Whereas the lowest shoot length was recorded at spacing 20 x 10cm,, 20x15cm 20x15cm and 30 x 10cm i.e 11.53cm, 12.17cm, 12.17cm and 11.45cm respectively. After 50 and 60 days of transplantation, a similar trend in increase in shoot length was observed in the plants of spacing 20x5cm and 30x5cm (Table 3 & 4) however on 60 days of transplantation the minimum growth was recorded only at spacing 30x 15cm (49.9cm). After 60 days no further increase in shoot length was observed in any spacing combination.

Table 2. Effect of spacing on various growth parameters of potato plantlets (Desiree) after 30 days of transplantation.

Spacing (cm)	Shoot length (cm)	No of leaves per plant	No of nodes	No of branches	No of leaves per branch
S1(30 x 15)	12.63bc	16.25abc	14.25abcd	7.25 a	4.50 cde
S2(30 x 10)	11.45 c	15.25 c	13.87 bcd	6.75ab	5.5 bc
S3(10 x 15)	14.08ab	17.00abc	15.00abc	4.52 d	5.25bcd
S4(20 x 5)	14.36 a	18.12 a	15.75 a	5.00cd	6.62 a
S5(30 x 5)	14.01ab	17.62ab	15.62ab	4.75cd	5.75ab
S6(10 x 5)	12.81abc	15.62 c	13.62 cd	5.62 bc	4.00 e
S7(20 x 15)	12.17 c	16.25abc	13.62 cd	5.87 abc	5.50bc
S8(10 x 10)	12.51bc	15.62 c	13.00 d	6.00abc	4.37 de
S9(20 x 10)	11.53 c	16.25abc	13.87bcd	4.12 d	5.50bc

Different alphabets with each mean value in a column indicates significant differences of mean value at P≤0.05

Numbers of Leaves per plant

The data pertaining to numbers of leaves presented in Table 1 revealed no significant difference (P>0.05 in number of leaves among various spacing. After 30 days (Table 2) showed that leaves emerge in maximum number at spacing 20x5cm that have a mean value of 18.12, followed as 17.62 cm from 30x5cm spacing.

The data pertaining to number of leaves after 50 days of transplantation showed a significantly higher average numbers of leaves at 30x15cm spacing i.e 24.62. (Table 3). A similar trend in average lead number was observed in plants of various spacing after 60 days (Table 4) and no further increase in leaves number was recorded after this period.

Number of nodes

No significant difference (P>0.05 was present in average number of nodes after 10 days of transplantation. However, highest mean no of nodes were recorded in spacing 20x5cm (11.0) (Table 1). After 30 days (Table 3) of transplantation similar trend in mean average number of nodes was observed.

Data after 50 days recorded for number of nodes revealed

that the plants of spacing 30x15cm and 20x5cm have maximum number of nodes i.e 21.87 & 20.25, respectively. Minimum numbers were recorded in 10x10cm spacing i.e 13.00 mean nodes per plant (Table 3).

At about 60 days of transplantation, maximum mean numbers of nodes was observed in 30x15cm spacing i.e 25.37 nodes (Table 4). After 60 days no change in the mean number of nodes were recorded

Number of branches

Branches emerge after 30 days of transplantation and the data pertaining to number of branches showed that the maximum mean number of branches were present in 30x15cm spacing (7.25). Minimum average number of branches i.e 4.125 were recorded at spacing 20x10cm (Table 2).

After 50 days the data regarding number of branches per plant (Table 3) showed the maximum average value i.e 9.25 and 8.00 at spacing 30x15cm and 30x10cm respectively. Whereas the minimum mean numbers of branches (4.896) were observed at spacing 10x5cm.

No significant difference was recorded in mean branch number after 60 days of transplantation except at spacing 30x15 cm which showed significantly maximum branches (14.75) (Table 4).

Table 3. Effect of spacing on various growth parameters of potato plantlets (Desiree) after 50 days of transplantation.

Spacing (cm)	Shoot length (cm)	No of leaves	No of nodes	No of branches	No of leaves per branch
S1(30 x 15)	35.66 de	24.62 a	21.87 a	9.250 a	6.500
S2(30 x 10)	37.68 bcde	21.50 b	18.87 b	8.000 b	5.250
S3(10 x 15	40.90abc	21.00 ab	18.37 b	6.875 b	6.625
S4(20 x 5)	43.96 a	23.00ab	20.25ab	5.875 b	6.125
S5(30 x 5)	42.92ab	21.25 b	18.62 b	6.000 b	5.875
S6(10 x 5)	41.70abcd	23.12 b	20.25 ab	4.896 c	6.000
S7(20 x 15)	33.21 e	21.75 b	19.25 b	7.000 b	6.500
S8(10 x 10)	35.91cde	21.37 b	18.75 b	6.375 b	6.250
S9(20 x 10)	35.40 de	21.87 b	19.25 b	5.960 b	6.125

Different alphabets with each mean value in a column indicates significant differences of mean value at P≤0.05

Number of leaves per branch:

Data pertaining to number of leaves per branch after 30 days of transplantation showed maximum average leaves per branch (6.62) were recorded at spacing 20x5cm followed by (5.75) at spacing 30x5cm. Whereas at spacing 30x10cm, 20x15cm and 20x10cm the same number of leaves per branch was recorded i.e 5.50 (Table 2).

Mean number of leaves per branch increases with time in all the spacing but no significant difference (P>0.05) was found after 50 days among all nine space combinations (Table 3). After 60 days of transplantation the data pertaining to number of leaves per branch revealed maximum average leaf number per branch at spacing 30x15cm i.e 10.12 (Table 4) while minimum average value (6.000) was recorded at spacing 10x5cm . After 60 days further growth was not observed in plants at any spacing.

Table 4. Effect of spacing on various growth parameters of potato plantlets (Desiree) after 60 days of transplantation.

Spacing (cm)	Shoot length (cm)	No of leaves	No of nodes	No of branches	No of leaves per branch
S1(30 x 15)	49.90 d	29.00 a	25.37 a	14.75 a	10.12a
S2(30 x 10)	53.28bcd	24.25 b	21.12 b	11.00 b	8.50 abc
S3(10 x 15	54.85 ab	24.87 b	22.62 b	10.00 b	6.25 ab
S4(20 x 5)	58.92 a	26.00 b	22.62 b	10.12 b	9.12 d
S5(30 x 5)	59.01 a	25.12 b	22.50 b	10.50 b	7.12 cd
S6(10 x 5)	57.01 ab	26.25 b	23.12 b	9. 12 b	6.00 d
S7(20 x 15)	54.55bc	24.62 b	21.37 b	9.12b	9.25ab
S8(10 x 10)	53.92bcd	24.37 b	21.25 b	10.7 b	9.75ab
S9(20 x 10)	50.50 cd	25.00 b	22.00 b	9. 75 b	8.37bc

Different alphabets with each mean value in a column indicates significant differences of mean value at P≤0.05.

Regarding tuber yield, the tubers were categorized into three types on the basis of weight i.e large > 20g; medium 10-20g; small < 10g (Fig. 2).

Tubers Numbers

Data regarding average tuber number revealed that large size tubers are formed in small number as compared to medium and small size tubers among all nine plant spacing combi-

nations (Table 5a) (Fig.2). The highest average tuber number (1.28; 2.25; 8.87) of all the three tuber size categories i.e large, medium and small tubers, respectively were recorded at spacing 30x15cm and 20x10cm (Table 5).

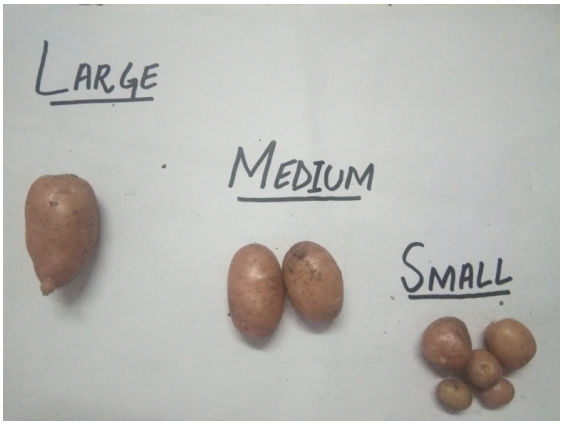

Figure 2. Tuber categories on the basis of weight i.e large > 20g; medium 10-20g; small < 10g.

Tubers Weight

No significant difference(P>0.05) was observed in all nine plant spacing combinations in case of average weight of large tubers (Table 5a). The highest average weight of large size tubers (28.7g) were recorded at spacing 30x15cm followed by 20x10cm i.e 27.54g. Analysis of variance revealed maximum average weight of medium size tubers (31.45g) at inter and intra row spacing of 20x15cm, followed by (27.87g) at 30x15cm (Table 5). In case of small size tubers the greatest mean average weight was recorded at spacing 20x10cm (33.49g) and 30x15cm (32.26g). However, in all the three tubers categories the lowest mean tuber weight was recorded at the plant spacing 10x5cm (Table 5a).

Tubers Diameter

The data for mean tuber diameter revealed that the maximum mean value (28.33 mm and 27.04mm) was obtained at spacing 30x15cm and 20x10cm, respectively for large size tuber category (Table 5b). Similar trend of maximum tuber diameter was also recorded in case of medium and small tuber categories. Whereas least average tuber diameter was recorded at spacing 10x5cm in all the three tuber categories (Table 5b).

Number of Eyes on tubers

Mean number of eyes per large tuber presented in Table 5 showed maximum numbers 5.62 at spacing 20x10cm followed by 30x15cm (5.50). No significant difference (P>0.05) was observed in average number of eyes on medium and small size tubers (Table 5b). Relatively tubers of all spacing showed average 4-5 eyes per tuber for medium and small tubers, respectively.

Average tuber yield per plant

A significant difference (P≤0.05) is noted on the average yield obtained in all nine spacing combination (Table 5b). The highest average tuber yield per plant (165.0g) was recorded at inter and intra row spacing of 30x 15cm (Table 5b), followed by spacing 20x10cm in which the mean total yield was 145.38g. The lowest yield (58.62g) was recorded at row and plant spacing of 10x5cm (Table 5b).

Table 5a. Effect of spacing on tuber size and yield of potato variety Desiree after harvesting.

Spacing (cm)	Tubers weight (g)			Tuber numbers		
	large	medium	small	large	Medium	small
S1(30X15)	28.71 a	27.87 ab	32.26 a	1.28 a	2.12 a	8.87 a
S2(30X10)	25.45 a	19.35 bc	29.10 ab	1.00 a	1.37 b	6.87 abc
S3(10X15)	27.54 a	18.06 c	27.97 ab	1.14 a	1.16 b	5.87 bc
S4(20X5)	22.70 a	19.95 bc	25.63 ab	1.00 a	1.42 b	6.50 abc
S5(30X5)	23.10 a	18.33 bc	21.63 ab	1.00 a	1.42 b	6.12 bc
S6(10X5)	21.95 a	15.78 c	19.70 b	1.00 a	1.12 b	4.75 c
S7(20X15)	24.25 a	31.45 a	26.14 ab	1.00 a	2.25 a	7.25 abc
S8(10X10)	23.13 a	20.75 bc	25.63 ab	1.00 a	1.37 b	6.50 abc
S9(20X10)	26.07 a	21.95 bc	33.49 a	1.20 a	1.42 b	7.75 ab

Different alphabets with each mean value in a column indicates significant differences of mean value at P≤0.05.

Table 5b. Effect of spacing on tuber size and yield of potato variety Desiree after harvesting.

Spacing (cm)	Tuber diameter (mm)			Number of eyes per tuber			Av. yield per plant (g)
	large	medium	small	large	medium	small	
S1(30X15)	28.33 a	22.97 abc	16.52 a	5.50 a	5.37 a	4.00 a	165.00 a
S2(30X10)	26.87abc	25.05 a	15.68 ab	1.87 b	5.87 a	4.25 a	100.63 cde
S3(10X15)	25.50abc	22.61 abc	15.55 ab	4.62ab	5.50 a	4.00 a	101.88 cd
S4(20X5)	25.15abc	22.27 abc	15.13 bc	1.87 b	5.12 a	4.12 a	77.875 de
S5(30X5)	25.30abc	21.62 bc	14.67 bc	2.37ab	5.62a	4.37 a	84.500 de
S6(10X5)	24.15 bc	20.26 c	15.31 bc	1.75 b	5.00 a	4.12 a	58.625 e
S7(20X15)	25.97abc	22.27 abc	14.33 c	3.25ab	5.37 a	3.87 a	128.88 abc
S8(10X10)	23.96 c	22.38 abc	15.12 bc	5.62 a	4.62 a	4.00 a	109.00 bcd
S9(20X10)	27.04 ab	23.91 ab	15.36 b	5.00ab	4.87 a	4.00 a	145.38 ab

Different alphabets with each mean value in a column indicates significant differences of mean value at P≤0.05.

Discussion

In potato crop, plants inter and intra spacing plays an important role on crop growth and productivity. An inappropriate spacing may lead to either too dense or too thin plant population resulting in yield reduction. In this study very densely populated plants (10 x 5, 20x5 and 30x5 cm) in terms of plant to plant distance with in a row (intra plant spacing) which was 5cm showed good vegetative growth in terms of shoot length, number of leaves etc. The result of the experiment was in line with the findings of Kifle Zerga *et al.,* (2017) who reported that densely populated plants showed intensive competition which leads to increase in plant height.

The effect of plant spacing observed on number of leaves and nodes was that maximum numbers appeared in widest spacing within plants. This may be due to availability of better nutritional components. This sought of information was reported by Idoko *et al*., (2017) in which the maximum number of leaves were seen at widest spacing, which gave it the ability to initiate more leaves as a result of enough above and below ground natural resources available to it. As the number of leaves increases, number of nodes also increased.

The number of branches per plant were considerably affected by spacing in our experiment. It was noticed that with the increase in spacing, increases the number of branches (14.75) at inter row spacing of 30 cm and intra plant spacing 15 cm, but at closer spacing, the mean number of branches per plants were found decreased. This may be due to the fact that plants transplanted at wider spacing have relatively less competition and they found more space to grow and produce more branches for absorption of light that enhances photosynthetic activity which resulted in good vegetative growth of the plants.

Regarding number of large size tubers maximum number was recorded at wider spacing 30 x 15 cm. This may be due to less competition within plants at wider space. Plants can absorb the sufficient nutrients and utilized them in producing the large size tuber.

This result is in consistency with the work of Tesfaey Getachew (2013) who confirmed that maximum number of large tubers in total tuber yield was increased with wider spacing among the plants. Similarly Yenagi *et al*., (2004), Essah *et al*., (2004) and Tafi *et al*., (2010) also confirmed the increased percentage of large size tubers at wider spacing as compared to narrow spacing. Narrow spacing leads toward decrease in numbers of large size tubers. Gulluoglu and Arioglu (2009) also have reported that mean tubers weight and tuber yield per plant significantly decreased as plants were planted closer due to increase in inter plant competition.

Regarding small size tubers category (small < 10g) more mean tubers number was observed at narrow spacing. This is because of high competition among the plants, plants were may be unable to get the required amount of nutrients from the soil due to dense vegetation and they were competing to attain height rather than storing the food in the tubers.

Mean average tubers yield per plant is also influenced by the plant spacing. By decreasing plant space, a significant reduction in yield was observed and lowest mean tuber yield per plant was recorded at 10 × 5 cm. Bhagirath (2013) also reported that plant spacing influence the vegetative growth and yield of potato tubers; wider the plants spaced, maximum will be the average yield.

Conclusion and Recommendations

It has been concluded from the results that inter and intra spacing of 30x15cm comparatively produces the highest yield. In the experiment, the spacing of 30x5cm, 20x5cm and 10x5cm lead to the maximum vegetative growth whereas the wider spacing 30x15cm leads to the maximum tuber yield.

Therefore, it has been recommended that the spacing of 30 cm between rows and 15 cm between plants is the best space combination for the transplantation of tissue cultured potato plantlets in the green house for maximum mini-tubers production. It has also been recommended that this plant spacing can also be tested for other plants produced through tissue culture for their transplantation on greenhouse benches.

Compliance with Ethical Standards

Conflict of interest

The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Author contribution

The contribution of the authors is equal. All the authors read

and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Consent for publication

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