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An improved method of preparing onion bulbs for the *Alium* test

MAŁGORZATA WIERZBICKA

Laboratory of Electron Microscopy, Institute of Botany, University of Warsaw,
Krakowskie Przedmieście 26/28, 00-927 Warsaw, Poland

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Abstract

The tests on storage and preparation of onion (*Allium cepa* L.) bulbs presented in this paper were performed in order to obtain the highest possible number of roots of similar length, which would be suitable for performing the *Allium* test. The results were subject to a detailed statistical analysis and allowed the following procedure to be recommended: 1) Store the bulbs at room temperature rather than in a refrigerator for two weeks before starting the experiments. 2) Do not use the biggest bulbs (over 80–100 g); use medium and small bulbs with the largest possible diameter of the reduced stem. 3) Just before starting the culture, wash the bottom part of the bulb, cut out the central part of the reduced stem and cut off the upper part of the bulb. At least 70% of bulbs prepared this way are expected to be suitable for cytological tests.

Key words: *Allium test, bulb, root growth, preparing bulbs, onion*

INTRODUCTION

The *Allium* test, introduced by Levan, is well known and often used (Fiskesjö 1979, 1981a, b, Grant 1982, Mandal and Basu 1981). Its main advantages are: easy availability and low cost of material — usually a commercially available multivaraity mixture is used (Lancaster and Collin 1981). The *Allium* test allows the observation of mitotic activity in many identical adventitious root tips where cells are relatively large and contain well discernible chromosomes.

While studying the effect of totally different agents: lead salts (Wierzbicka 1984) and an anticancer drug Ledakrin (Antosiewicz 1987) on cell division it was found that the uptake of these compounds is very fast. Hence, the reproducibility of experiments carried out with the *Allium* test can be strongly influenced by the amount of substance per unit mass of root

that is provided in the incubation chamber. It is very probable that this also is the case in studies with other compounds, particularly when they are supplied in aqueous solutions and not in the culture medium, i.e. without other ions. This means that large numbers of onion bulbs with a similar number of roots of the same length are necessary in order to perform *Allium* test experiments. In practice, such material is very difficult to obtain.

Different methods of onion selection for experiments, and various methods of mechanical damage (preparation) are traditionaly used. The only extensive published analysis of these methods is given in the paper by Schaede (1927). Therefore, testing and selecting optimal and statistically confirmed methods of preparation of onion bulbs for cytological experiments seemd worthwhile.

The following specific questions were answered:

1. How to prepare bulbs before growing?
2. At what temperature should bulbs be stored just before growing?
3. What should be the morphological features of the selected bulbs?

MATERIAL AND METHODS

Since the worst period for onion bulb root growth is from August to February (dormant bulbs), the experiments were performed in November for two successive years. A multivariety mixture of the commercially available onion *Allium cepa* L. was used. The bulbs were grown in 250 cm^3

Table 1

Methods of preparation of onion bulbs in different experimental groups

Bulb preparation		Group number									
		1	2	3	4	5	6	7	8	9	10
Reduce stem damage*	brushing	+	+	+	+	+	+	+	+	+	
	pricking with a needle 10 times to a depth of 5–7 mm		+				+				
	cutting a 1 mm thick slice from the reduced stem			+				+			
	cone-like hollowing out of the center of the reduced stem (depth 5 mm, diameter 5 mm)				+				+	+	
Upper part damage	cutting off						+	+	+		
	removal of two external scales									+	

* The stem part of a onion bulb is reduced to a plate with short internodes, no pith, and congested traces of leaves and adventitious roots (Esau 1965). This plate is called the "reduced stem" in the text.

jars for 5 days at room temperature in tap water. A set of 10 bulbs was grown in one experiment. After 5 days, all roots were cut off, fixed in 10% formaldehyde and their length was measured.

BULB PREPARATION

One hundred bulbs, stored at room temperature before the experiments, were divided into 9 groups and prepared in different ways, summarized in Table 1. The method of preparation of the upper part of bulbs is shown in Fig. 1.

STORING TEMPERATURE

Bulbs were stored for 10 days at 0°C (20 bulbs) and 37°C (20 bulbs) (Table 2). Then half of them were prepared just before growing as group 8 (Table 1), the remaining 2 × 10 bulbs were left untouched (Table 2).

Table 2

Storage temperature and subsequent treatment of onion bulbs

Treatment		Group number			
		11	12	13	14
Temperature 0°C	unprepared	+			
	prepared		+		
Temperature +37°C	unprepared			+	
	prepared				+

MORPHOLOGICAL FEATURES

60 bulbs, stored at room temperature, selected for their extremal shapes and dimensions were grown for 5 days without preparation. Later, the following parameters were measured for these 60 bulbs as well as for 140 bulbs grown in the two previous experiments, in order to establish correlations between bulb anatomical structure and the subsequent growth of their roots. The parameters are the diameter of the widest, the distance between this part and the reduced stem (see footnote to Table 1), the diameter and the height of the reduced stem. Using these data, the volume of the lower half of the bulb, the volume of the reduced stem and the shape coefficient (elongation ratio) were calculated. The data obtained were compared with the number of roots, their average length and time needed for their emergence.

RESULTS AND DISCUSSION

BULB PREPARATION

The influence of various mechanical damage on the time needed for roots to emerge (Table 3), number of roots (Table 4), and their length was estimated with the help of one-way analysis of variance (Armitage 1971). In all three cases, the level of significance α was less than 0.01. This means that the mechanical damage applied stimulated the onion root growth in a statistically significant way. With the help of the Scheffe method (Armitage 1971) the hypotesis on the differentiation of various averages was verified. It was shown (at the level of significance $\alpha < 0.05$) that the roots emerge earlier and are more numerous after hollowing out the reduced stem and cutting off the upper part of the bulb (group 8, Tables 3, 4). On the other hand, in a statistically significant way the

Table 3

Time needed for roots to emerge in different experimental groups during onion bulb growth (10 bulbs were grown simultaneously in each group)

Day	Number of bulbs with emerged roots in different experimental groups													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	5	8	8	5	4	8	9	7	9	2	0	3	0	7
2	8	9	10	8	5	8	9	10	10	2	2	6	1	10
3	8	10	10	9	5	10	10	10	10	3	2	10	5	10
4	8	10	10	10	5	10	10	10	10	4	4	10	7	10
5	8	10	10	10	5	10	10	10	10	5	4	10	7	10

roots were longer (Table 4) after hollowing out the center of the reduced stem (group 4), pricking the stem with a needle and cutting off the upper part of the bulb (group 6), cutting out a thin slice of the reduced stem and cutting off the upper part of bulbs (group 7), hollowing out the center of the reduced stem and partial or total cutting the upper part of the bulb off (groups 8, 9).

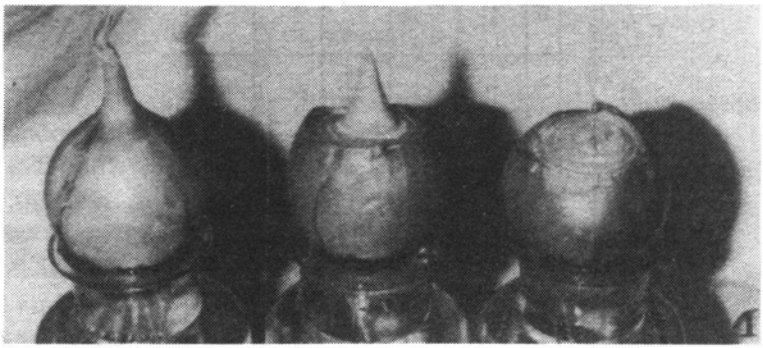
Pricking the reduced stems is, however, not the proper method of bulb preparation, because it leads to leaking of juice from onions and causes chromosome aberrations during cell division in root tips (Keck and Hoffman-Ostenhof 1956). Also shallow cutting off the reduced stem and its sharp brushing, although treatment of this kind was used in some laboratories (Fiskesjö 1981a, b, Grant 1982), is not the proper method,

Table 4

Characteristic features of the root system of onion bulbs in different experimental groups after 5 days of growth

Features	Group number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Average number of roots in one bulb	37	35	39	40	16	37	34	53	36	22	13	44	28	52
Average length of roots in one bulb, cm	1.5	2.0	2.1	2.4	1.7	3.7	2.5	4.0	3.7	0.8	1.1	2.6	1.4	2.8
Roots with deformed tips, %	31.9	14.7	27.5	15.5	35.2	13.9	26.4	12.0	10.1	5.8	14.1	21.3	5.4	13.7
Number of bulbs with more than 40 roots	5	5	6	4	1	4	5	7	4	3	0	7	3	7
Number of bulbs with average length of roots over 3.5 cm	0	0	0	1	0	7	1	7	5	0	0	1	0	0

PLATE I



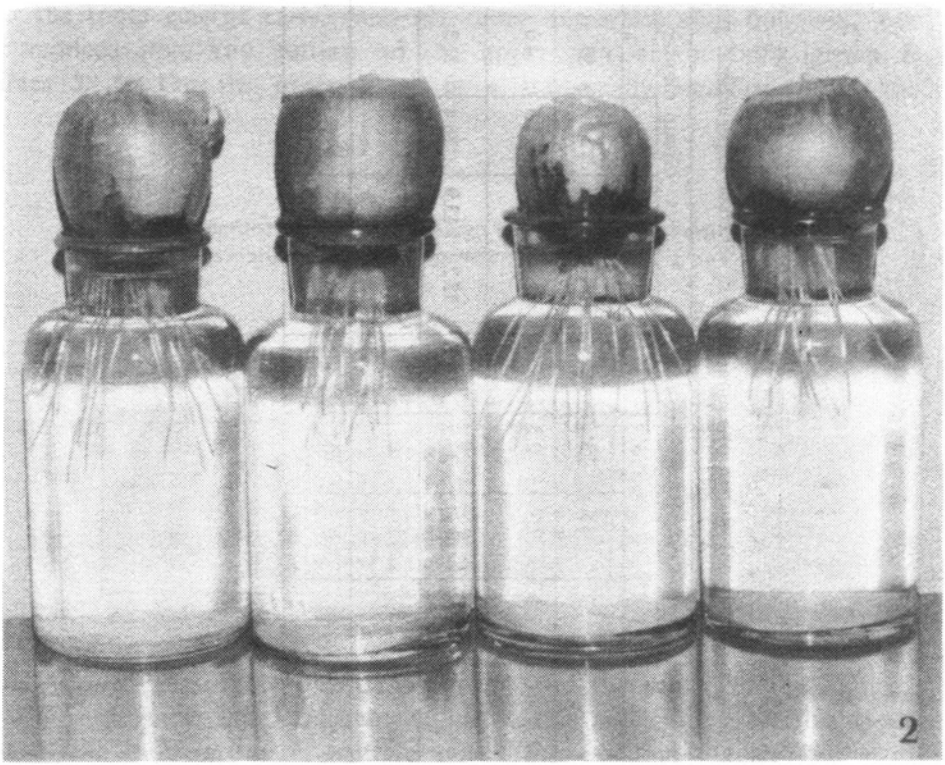


Fig. 1. Upper parts of the onion bulbs prepared in various ways in order to obtain uniform root growth

Fig. 2. Induction of root growth in onion bulbs that were initially (for 5 days) grown after removing the upper part of each bulb without damaging the reduced stem (group 5, Table 1). Since roots did not emerge, the center of the reduced stem was hollowed out. The picture shows the roots which emerged after further 5 days of growth

since it leads to a larger number of roots with deformed tips (from 26.4 to 35.2%, Table 4). Such roots were always short (1–2 cm), often curved, with dark ends and without visible apical meristems.

In order to determine what kind of damage led to the largest number of roots of equal length, a coefficient of variation analysis (Armitage 1971) of their length was performed. It was shown that any kind of preparation of bulbs led to a decrease of differences in number and length between particular bulbs within each experimental group, comparing with the differences between the bulbs in the control group. The coefficient of variation was the smallest (8.9%) after hollowing out the reduced stem and cutting off the upper part (group 8), whereas in the control group it was 87.3%.

Eventually, the best and statistically proved effect of growth stimulation was achieved after cutting off the center of the reduced stem and removing the upper part of each bulb (group 8). After such damage, the roots emerged on the second day (Table 3), their mean number was 53 in each bulb (Table 4) and they attained an average length of 4 cm (Table 4). The smallest differences between bulbs in each group were achieved with this method. Therefore, this is the best way to perform cytological experiments, since it leads to 70% of bulbs that have over 40 roots with an average length of over 3.5 cm (Table 4). It should be noted that the application of all of the methods of bulb preparation gave better results than those obtained for the control group. The only exception was group 5 in which the upper part of the bulb was cut off and the reduced stem only brushed. Not only did this not stimulate the roots to grow, but the root growth stimulation was limited to 50% of bulbs, the roots were less numerous and exhibited a slower growth rate (Tables 3, 4). If later the center of the reduced stem was hollowed out in the bulbs in which the roots had not emerged despite 5 days of keeping in water (the 50% mentioned above), numerous roots emerged rapidly (Fig. 2). Therefore, it should be assumed that significant damage of the reduced stem is necessary if, by cutting off the upper part, growth by hormonal stimulation connected with tissue injury is to be attained. This indicated that the reduced stem tissue isolates primordial adventitious roots from water, in agreement with the suggestions of Schaede (1927).

BULB STORAGE

Storage temperature is known to strongly influence the rest period (Vegis 1964). Nevertheless, a question remaining to be answered was if the storage temperature just prior to the experiment influenced the subsequent root growth.

The effect of temperature (groups 8, 10 — room temperature; groups 11, 12 — 0°C; groups 13, 14 — 37°C) on time needed for roots to emerge

(Table 3), the number of roots (Table 4) and their length (Table 4) were estimated with the help of the two-way analysis of variance (Armitage 1971) $\alpha < 0.01$. It was found that the storage temperature had a statistically significant influence on the root length and time after which they emerged. The bulbs stored at 0°C (groups 11 and 12) had the smallest root growth (Tables 3, 4). Also, statistically significant differences in length and time of root emergence between prepared and unprepared bulbs were found. Prepared bulbs (groups 8, 12, 14) always had longer and earlier roots than the unprepared ones (groups 10, 11, 13, respectively). In addition, the overlap of temperature and preparation factors had a statistically significant effect on the length and time of emergence of bulb roots.

Quickly emerging and, eventually, the longest roots grew from the prepared bulbs that were stored at room temperature (group 8). Storage temperature, however, did not have any statistically proven effect on the number of roots. In this case the method of preparation was the only significant factor.

So, it can be concluded that storage conditions just before experiment affect root emergence and their length. Room temperature is the optimal temperature for storage. Therefore, it is practical to advise that onion bulbs should be removed from the refrigerator a dozen or so days before the experiment starts.

BULB SELECTION

The third aspect of this study was to determine what kind of bulbs are the best for studies. The result of Ward (1979) show that successful natural onion storage depends on bulb size: the number of germinating bulbs increase with the bulb size. This observation suggests that selection of bulbs with similar dimensions can be meaningful for the *Allium* test — this was observed in their own test experiments by some authors (Fiskesjö 1981a, b, Grant 1982).

For the experiments reported, bulbs were selected according to extreme dimensions with weight 21.0–171.5 g and volume of the lower half 9.1–100.1 cm^3, volume of the reduced stem 350–3993.5 mm^3 and the reduced stem diameter 7.4–21.9 mm. One-way analysis of variance of the number and length of roots ($\alpha < 0.05$) did not show statistically significant differences in root growth between the biggest and smallest bulbs. The analysis of correlation coefficients (Armitage 1971) between weight and volume of the particular parts of the bulbs, and the number and length of roots did not prove the existence of any correlations. The only exception concerned the time after which the roots emerged; among small bulbs with an average weight of 29.5 g, 80% of bulbs had roots after 5 days, as opposed to 30% among the largest bulbs (average weight 145.2 g).

Statistical analysis with the help of the Mann and Whitney distribution of "U" (Armitage 1971), which verifies if two samples represent the same population, showed that a significant difference ($\alpha < 0.05$) exists between the smallest (21–30 g) and the largest (106.5–171.5 g) bulbs.

A similar analysis was performed with bulbs prepared before growing (groups 1–14). The weight of these bulbs was 30–75 g, the volume of the reduced stem 550–2173 mm^3, the diameter of the reduced stem was 8.5–15.5 mm. A small positive correlation was found (correlation coefficient $r = 0.6$ for $\alpha < 0.01$) between the diameter of the reduced stem and the number of roots only in the case when the central part of the reduced stem was hollowed out (groups 4, 8).

Finally, the data presented indicate that selecting onion bulbs with similar morphological features is not necessary for cytological experiments. Such selection will not reduce differences between root growth in different onion bulbs although roots grow slightly faster and are more numerous in small onion bulbs (20–30 g) with a large diameter of the reduced stem.

In addition one should take into account that maleic hydrazine (Chroboczek 1970), is often used to extend the rest period of onion bulbs *Allium cepa* L. This compound strongly inhibits root growth and leads to chromosome aberrations (Heindorff and Rieger 1984). The formation of bulbs during onion growth depends on many factors (Terabun 1981, Ohkubo et al. 1981). Nowadays, fungicides which affect onion bulb formation are often prophylactically applied (Giovanetti and Ries 1980). The period of onion harvesting also affects the rate of the subsequent root growth (Ward 1979). Such treatments may have an essential effect on the subsequent observations of cell division in onion roots. Because of the possibility that different onion bulbs have been treated by farmers differently, all the onion bulbs needed for experiments should be purchased at the same time and stored in the same place; the whole experiment should be performed in the shortest time possible. The bulb outer brownish scales should be removed and the bulbs very carefuly washed before preparation.

The preparation of onions should be applied for short-term (a few day long) experiments. When planning longer experiments, one should take into account Schaede's (1927) results; he performed an approximately two week long culture of prepared and unprepared onion bulbs simultaneously comparing the fresh weight of their roots. He found that although for the first 13 days the roots of prepared onion bulbs were heavier than the roots of unprepared onion bulbs, after a longer time these proportions were reversed.

The conclusions from the present report are as follows. When preparing experiments using the *Allium* test one should store onion bulbs at room temperature. Subsequently, one should discard the largest bulbs (over 80–100 g);

after careful but delicate washing of the lower half, the center of the reduced stem should be hollowed out, and the upper part of the bulb should be removed. In a series of onion bulbs prepared in this way one can expect that 70% of the onion bulbs should be suitable for cytological tests, even during the state of repose.

One should note, however, that the treatments tested and described in the present paper have not led to an ideal uniformization of the number of roots and their growth rate. No two onion bulbs with the same root system were found among 200 onion bulbs carefuly studied in this work. Only a significant acceleration of root growth and the reduction of differences between the roots of particular onion bulbs were achieved.

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Ulepszona metoda przygotowania cebul do testu Allium

Streszczenie

Po przeprowadzeniu wielu prób różnych sposobów przechowywania i preparowania cebul *Allium cepa* L., w celu uzyskania jak największej liczby korzeni jednakowej długości, nadających się do przeprowadzenia doświadczeń testem *Allium* i szczegółowej analizy statystycznej, zalecam w praktyce:

1. Przez okres 2 tygodni przed doświadczeniem cebule przechowywać w temperaturze pokojowej, a nie w lodówce.
2. Do doświadczeń nie używać cebul największych (powyżej 80–100 g). Pozostawić cebule średnie i najmniejsze o możliwie największej średnicy piętki.
3. Bezpośrednio przed hodowlą, po obmyciu dolnej połowy cebuli wyciąć środek piętki, oraz ściąć górną część cebuli (szyjkę).

W serii cebul tak przygotowanych można spodziewać się co najmniej 70% cebul nadających się do prowadzenia testów cytologicznych.