Changes in the concentration of sugars in developing ovules

Zmiany w stężeniu cukrów w rozwijających się zalążkach

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INTRODUCTION

In his previous papers (1960a, c) the author presented the results of his studies on the osmotic value of the central vacuolar sap in developing ovules. It has been established that this value increases with the age of the ovules in all examined species, till it attains a determined level and subsequently drops and remains on a more or less constant level. In order to explain this phenomenon, a hypothesis was advanced (in the paper 1960a) that the fluctuations of the osmotic value of the central vacuolar sap during the development of the ovules are the result of two processes; the first consists in the transfer of nutrative substances (amino acids, sugars) to the ovule from the vegetative organs (P r o k o f i e v and S o b o l y e v 1957, Z h d a n o v a, L e b e d y e v, C h v i z h 1960), the second is the synthesis of storage substances of low osmotic activity (protein, polisaccharides) in the developing endosperm (K o b l e t 1940, E v a n s 1941, K o l o b k o v a 1958, G r z e s i u k and R e j o w s k i 1960). Further investigations on the changes of the amount of carbohydrates and nitrogen compounds occuring in the central vacuolar sap during the growth and development are necessary to corroborate or disprove this hypothesis.

It should be noticed that the above mentioned two groups of compounds were and will be essential for cultures in vitro both of the embryos and of the nuclear endosperm (S a n d e r s, B u r k h o l d e r 1948, C u t t e r jr. and W i l s o n 1954, B a j e r 1955, 1957, S t r a u s 1960).

This study has been carried out in order to:

1) determine the concentration of soluble sugars in the sap of the central vacuole during the growth and development of the ovules;

2) discover any dependence between the concentration of sugars in the sap, the water content and the synthesis of storage compounds in developing ovules.

MATERIAL AND METHOD

The ovules of *Clivia* sp., *Haemanthus Katharinae* and *Haemanthus albiflos* provided the material used in these studies. The criterion adopted for the estimation of the developmental stage of the ovules and the method elaborated for the measurements of their size were described in the previous papers (1960a, c).

The following procedure was adopted for the extraction of the vacuolar sap. An isolated ovule was punctured with a glass needle and pressed delicatly with two fingers in order to force the sap out. The drops of clear sap were collected in a previously prepared sterile pipette graduated to 0,001 ml.

The micro-method of S o m o g y i (1945) modified by W i l l i s and Y e m m (1955) was applied for estimations of the sugar concentration in the sap of the central vacuole. The amount of applied reagents, the preparation and storage procedures were the same as those recommended by the above mentioned investigators. The author finds it advisable to notice that the copper reagent and the sugar samples were warmed in a water bath (100°C) for 21 minutes and 15 minutes respectively. Both the copper reagent and the sugar samples were cooled for 3 minutes in tap water (not running) before further use. The time of warming and cooling the samples was observed with accuracy to 2 seconds. A blank test was made simultanously with the main test both
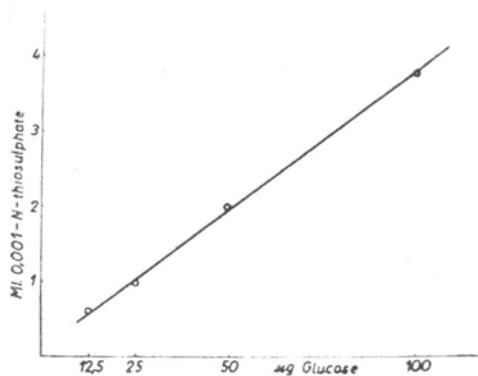

Fig. 1. Relation between the concentrations of the standard dilution of glucose in µg/ml and the amount of 0,001-N-Na$_2$S$_2$O$_3$ in ml necessary for titration

during calibration and estimation of sugars in the sap of the central vacuole. Calibration was carried out by means of two standard solutions of sugars: glucose and saccharose. The results of calibration by means of glucose solution are shown in Fig. 1. They indicate that the relation

between the sugar content in the sample and the amount of 0.001-N-$Na_2S_2O_3$ utilized for its titration is exactly linear in the examined range of glucose concentration (12.5 — 100 μg). An analogous relation was obtained for the standard solution of saccharose, though, the amounts of 0.001-N-$Na_2S_2O_3$ utilized for titration of identic doses of saccharose and glucose, were somewhat smaller. This is in conformity with the results obtained by Willis and Yemm (1955). In the range 12.5 — 100 μg with the standard solution of glucose and saccharose the error of estimation did not exceed 1 or 2 μg respectively. For estimations, the solution which contained the sugars of the central vacuolar sap was prepared according to the Yemm and Willis (1954) prescription. Before analysis this solution (containing sugars, amino-acids, protein and other compounds and cleared with aluminium hydroxyd cream) was centrifugated for 20 minutes at 5000 r.p.m. The supernatant solution, the volume of which was 0.5 — 1 ml (in dependence on the stage of development of the ovules) provided the material for the estimation of sugars; they were determined in the following order: 1) reducing sugars, 2) all sugars present in the solution (after hydrolysis). The hydrolysis of non reducing sugars was performed by applying 0.2-N-H_2SO_4 for 10 minutes in a water bath at 100°C. The non reducing sugars constituted the difference between the total amount of sugars and reducing sugars.

The amounts of reducing sugars were calculated from the standard curve obtained for glucose. whereas total sugars were determined from the standard curve obtained for saccharose. The results of estimation of sugars are shown in Fig. 2, 3 and in Table 3. They are given in mg for 1 ml sap of the central vacuole.

The water content in the ovules was determined by the weighing method. Isolated ovules were dried at 105°C to a constant weight. The material for examination was collected between 7 and 9 a.m. Examinations were performed in Summer 1960.

RESULTS

Clivia sp. The results of determination of soluble sugars in the sap of the central vacuole in the ovules of this plant species are shown in Fig. 2 (curve I — reducing sugars, II — all soluble sugars after hydrolysis, III — non reducing sugars). The concentration of reducing sugars in the sap of 9 days old ovules is 20 mg/ml sap and increases gradually during the further growth of the ovules and in 17 days old ones it attains 28 — 29 mg/ml sap. Subsequently it slightly drops (24 days old ovules) and finally it attains the value 40 mg/ml sap in 43 days old ovules. In still older ones a rapid drop of reducing sugars to 12 mg/ml

sap (50 days old ovules) was observed. In ovules 53 — 54 days old the concentration of these sugars is slightly greater in ccmparison to 50 days old ones and is 20 mg/ml. The total concentration of soluble sugars in the central vacuolar sap (curve II) in 9 — 24 days old ovules increases
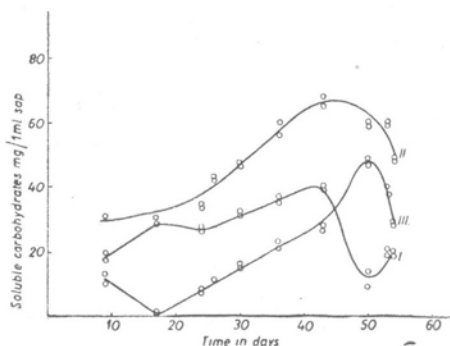

Fig. 2. *Clivia* sp. Changes in the concentration (mg/ml) of sugars in the sap of the central vacuole in dependence on the age of the ovules; abscissae: days from the moment the perianth dropped

(Curve *I* — reducing sugars, *II* — all soluble sugars after hydrolysis, *III* — non reducing sugars)

slowly from 30 till 35 mg/ml sap. The total amount of soluble sugars in the sap of ovules in the age of 24 and 43 days increases rapidly from 35 to 65 mg/ml sap. In older ovules (over 43 days) a decrease to 50 mg/ml of the total amount of soluble sugars in the sap of the central vacuole (54 days old ovules) is observed. The concentration of non-reducing sugars in 9 days old ovules is 11 mg/ml sap, in older ones (17 days old) it decreases to 0, fig. 2 (curve III). In ovules from the 17 to the 50th day the concentration of non reducing sugars in the sap of the central vacuole increases almost frcm 0 to 47 mg/ml sap. A lower concentration of non-reducing sugars in the sap (39 — 30 mg/ml) is characteristic of ovules 53 to 54 days old.

The percentage of water content in relation to fresh weight in 9 — 43 days old ovules fluctuates between 91 — 93% (Tab. 1), the lowest water content (about 91%) was found in ovules in the 9th and 43rd day. A slow decrease of water content from 91 to 88% is characteristic of older ovules (43 — 87 days). Within 78 days (between the 9th and 87th day) the water content calculated for 1 ovule increase frcm 21.5 to 1331 mg. In this range of time one may distinguish two periods of intensive increase of water content. During the first period (9 — 17 day) the water content increases about 5 times. During the second one (38 — 43 days old ovules) the water content becomes about twice as great.

During growth and development the dry weight of 1 ovule rises from 2 mg (9 days old ovules) to 178 mg (87 days old ovules). Similarly to the increase of water content, we can distinguish two periods of intensive rise of the dry weight of the ovules (in 17 — 26 and 38 — 43 days old ovules).

Table 1

Clivia sp. — Water content of the ovules and their dry weight in dependence on their age (days after the perianth dropped)

Days	Water content % fresh weight	Water content 1 ovule in mg	Dry weight 1 ovule in mg
9	91.5	21.5	2.0
17	93.0	100.6	7.5
21	92.6	142.2	11.3
26	92.2	251.0	21.2
27	92.6	224.2	18.0
30	92.9	227.0	17.2
34	93.0	311.4	24.4
38	93.0	250.8	19.0
43	91.1	446.4	43.8
50	90.4	682.8	72.3
56	90.4	701.0	74.9
65	89.8	1061.6	121.4
75	88.4	990.0	129.4
87	88.2	1331.3	178.4

It results from the comparison of the results presented in Table I and Fig. 2 that the concentrations of total sugars, reducing sugars and non reducing sugars increase in general concomitantly with the increase of water content calculated for 1 ovule (ovules in the age of 9 — 43 days). A decrease of concentration of total and reducing sugars and a further decrease of water content calculated for 1 ovule was observed in older ovules (43 — 54 days old). The percentage of water content in these ovules in relation to fresh weight drops concomitantly, whereas the amount of non reducing sugars increases still for a time (50 days old ovules) and subsequently drops. The highest content of water calculated for 1 ovule was observed, for an 8 days long period, in 9 — 17 days old ovules in which reducing sugars were preponderant. Another maximal value of water content calculated for 1 ovule was found in 38 — 43 days old ovules i.e. at the age when the concentration of total sugars and reducing sugars in the sap of the central vacuole attains its maximum. In this period reducing sugars intensively change into non-reducing compound sugars.

Two distinct stages of intensive rise of dry weight can be distinguished a) in 17 — 26 days old ovules, when the concentration of total sugars in the sap of the central vacuole increases slowly and b) in 38 — 43 days old ovules when the maximal value of the concentration of sugars and reducing sugars is attained, but the concentration of non reducing sugars still increases (50 days old ovules).

Haemanthus Katharinae. The results of sugar estimations in the sap of the central vacuole in ovules of *H. Katharinae* are shown in Fig. 3. These results are similar to those obtained for *Clivia* sp. with the difference, however, that changes in the sugar concentrations proceed at
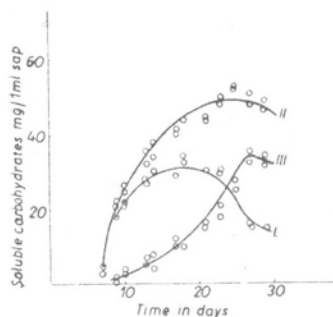

Fig. 3. *Haemanthus Katharinae.* Changes in the concentration of sugars in the sap of the central vacuole in dependence on the age of ovules. Abscissae: days from the moment the perianth wilted

(Curve *I, II, III* see Fig. 2)

a more rapid rate than in ovules of the latter species. The amounts of different sugars (reducing, total and non reducing sugars) are lower than in the case of *Clivia* sp.

Table 2

Haemanthus Katharinae — Water content of the ovules and their dry weight in dependence on their age (days after the perianth wilted)

Days	Water content % fresh weight	Water content 1 ovule in mg	Dry weight 1 ovule in mg
7	91.9	13.9	1.2
11	92.7	22.2	1.7
12	89.7	28.5	3.3
13	89.3	29.5	3.6
16	90.9	35.1	3.5
18	90.6	48.4	5.0
18	90.7	45.0	4.6
21	90.7	56.3	5.8
23	90.2	75.8	8.2
27	91.3	85.3	12.2
28	90.7	124.6	12.7
29	90.3	110.5	11.9
32	90.4	142.4	15.1
32	90.2	145.0	15.8
33	90.1	136.8	15.1
36	89.8	151.1	17.1
43	89.3	214.0	25.6
45	88.7	232.9	29.8
48	87.3	263.8	38.5
53	86.8	302.9	46.2
54	86.6	290.0	44.9

The changes in water content and dry weight of the ovules during their growth and development are presented in Table 2. The water content of younger ovules (percent of fresh weight) fluctuates about 90.5% (7 — 33 days old ovules). A slow decrease from 90% to 86% (33 to 54 days old ovules) is characteristic of older ovules. The water content calculated for 1 ovule in the age 7 — 54 days rises from 13.9 to 290 mg, whereas, the dry weight of one ovule increases from 1.24 to 44.9 mg.

Haemanthus albiflos. Neither the water content nor the dry weight in the ovules of this species were determined. The results of sugar estimations in the central vacuolar sap are assembled in Table 3 according to the size of the ovules. In ovules of the size 2.5 × 4.4 mm the concentration of the reducing sugars is the highest (30 — 31 mg/ml sap) and decreases gradually as the ovules grow older to 16 — 17 mg/ml sap (size of ovules 5.4 × 7.3 mm). In ovules 2.5 × 4.4 mm in size the concentration of total sugars in the central vacuolar sap fluctuates about 38 — 40 mg/ml sap and increases gradually till 64 — 67 mg/ml sap (ovules 4.5 × 6.6 mm) and then drops to 55 — 56 mg/ml sap (ovules 5.4 × 7.3 mm). Concomitantly with these changes, changes of non reducing sugars take place in the sap of the central vacuole; the amounts of these sugars gradually increase to 44 — 48 mg/ml sap (ovules 4.5 × 6.6 mm) and then drop to 39 mg/ml sap (ovules 5.4 × 7.3 mm).

Table 3

Haemanthus albiflos. Concentration of sugars in the sap of the central vacuole in dependence on the size of the ovules

Size of ovule in mm	Free reducing sugars mg/ml	Total soluble carbohydrates (after hydrolysis) mg/ml	None reducing sugars mg/ml
2.5 × 4.4	30.000	38.333	8.333
	31.389	40.556	9.167
3.3 × 5.1	23.750	48.000	24.250
	19.625	45.000	25.375
4.2 × 6.2	19.000	58.500	39.500
	19.000	56.500	37.500
4.5 × 6.6	19.250	67.000	48.750
	17.500	64.000	44.750
5.4 × 7.3	16.375	55.500	39.125
	17.000	56.500	39.500

DISCUSSION

The determination of the sugar content in the sap of the central vacuole during the growth of the ovules belonging to the three examined

species (*Clivia* sp., *Haemanthus Katharinae* and *Haemanthus albiflos*) yielded similar results. It is therefore reasonable to restrict the discussion to one species only e.g. *Clivia* sp.

Considering that the size of the central vacuole increases in ovules 9 — 40 days old (R y c z k o w s k i 1960c), one may see from the results shown in Table 1 that the rise of the concentration of total sugars from 30 — 65 mg/ml sap (Fig. 2) is connected with an inflow of water and other organic and inorganic compounds from vegetative organs of the plant to the ovule.

During the further growth and development of the ovules (from the 43rd to the 54th day of age) the total concentration of sugars decreases in spite of the rise of the water content calculated for 1 ovule. This is, however, only an apparent discrepancy, as, at the same time, a drop of percentage of water content in relation to fresh weight of the ovules was observed. This intensive increase of dry weight in ovules between the 17th and 26th day and 38 — 43 days old ones is connected not only with the increase of sugar concentration in the sap of the central vacuole. The results refering to the concentration of sugars in the sap of the central vacuole indicate that the absolute amounts of the three groups of sugars change in the course of growth and development of the ovules and the synthesis of non reducing sugars takes place at the cost of reducing sugars and in turn, starch is formed at the cost of non reducing sugars.

The obtained results are, as a rule, in conformity with those obtained by other investigators for whole wheat and corn grains. K o b l e t (1940) established that the sugar content in a wheat grain attained 2.2 g for 1000 grains on the 13th day following the beginning of blooming. Subsequently it gradually decreases till on the 35th day it was only 0,9 — 1,2 g, whereas, the content of protein and specially of starch increases concomitantly. E v a n s (1941) examined the chemical composition of a developing corn grain after silking and found that the content of such sugars as sucrose and dextrose expressed on dry weight basis, amounts to 5.24% and 3.8% on the 15th day. It decreases with age and on the 57th day it is 2.33% and 0.74%. The starch content in a grain on the 15th day is 22.8% and increases rapidly with its age and on the 36th day it attains a constant value 71.8%. There is a difference between the results presented in this paper and those obtained by K o b l e t (1940) and E v a n s (1941), but it is caused by the fact that these investigators experimented on an older material than the author (K o b l e t the 13th day following blooming and E v a n s the 15th day after silking). Thus, most probably, their first estimations of sugar content were performed at a time when the sugar content in a wheat

and a corn grain attains its maximal value. The results of estimation of water soluble sugars in a corn grain obtained by G r z e s i u k and R e- j o w s k i (1960), may be regarded as an argument suporting this divergence of results. They have established that the sugar content in a corn grain on the 12th day after anthesis is 28% on dry weight basis, on the 17th day it exceeds 38% and subsequently drops with the age of the grain to 4% (the 56th day after anthesis). The fact that K o b l e t (1940) and E v a n s (1941) used whole grains, whereas the author made his experiments on the central vacuolar sap, might have had a certain influence on the divergence of results.

The author prepared a supplementary graph (Fig. 4) permitting a more detailed analysis of changes of the physical and chemical pro- perties shown by the central vacuolar sap during the growth and
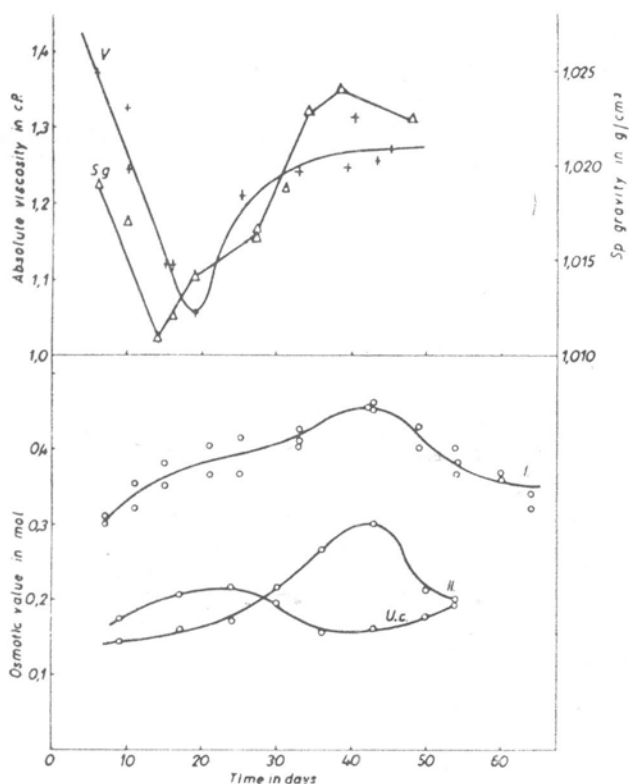

Fig. 4. *Clivia* sp. Abscissae: time in days. S.g. — specific gravity of the sap of the central vacuole

V — viscosity of the sap. Curve *I* — osmotic value of the sap (see paper 1960c). Curve *II* — osmotic value of the sap calculated from the sum of the concentrations of reducing and non- -reducing sugars in the sap of the central vacuole. U.c. — undetermined compounds in the sap of the central vacuole i.e. difference between curves *I* and *II*

development of ovules of *Clivia* sp. In this graph are shown the curves representing the osmotic value, the viscosity, the specific gravity of the central vacuolar sap and curve II which results from expressing the content of reducing and non reducing sugars in terms of osmotic value (in moles per liter). The curve denoted on the graph U.c. (undetermined compounds) represents the difference between the osmotic value, shown in Fig. 4 as curve I (R y c z k o w s k i 1960c, *Clivia* sp. curve I) and the osmotic value of the central vacuolar sap shown in Fig. 4 as curve II. An analogous curve was obtained also for the sap of the central vacuole in the ovules of *H. Katharinae*. It should be noticed that nitrogen compounds (K o l o b k o v a 1958, P o n t o v i c h, K a r i m o v 1960) are most probably the principal compounds shown in Fig. 4 as curve U.c. One may see from Fig. 4 that the specific gravity and the viscosity of the sap of the central vacuole in young $(4 - 5$ to 19 days old) ovules decrease (the causes of this phenomenon are explained in paper R y c z k o w s k i 1960 d) and subsequently rises till more or less constant values are attained $(39 - 48$ days old ovules). The specific gravity which is the resultant of the concentrations of all substances present in the sap of the central vacuole, begins to increase at an earlier age of the ovules (14 days old ovules) than the viscosity (19 days old ovules) because the latter one is determined first of all by protein substances, which begin to accumulate in the sap at an older age. An intensive increase of dry weight was observed to occur twice (in $17 - 26$ and $38 - 41$ days old ovules). The first rise took place at the time when an increase of specific gravity, viscosity and concentration of sugars and undetermined compounds (Fig. 4) was observed in the sap of the central vacuole. The second period of an increase of dry weight coincides with the moment when the specific gravity, viscosity and concentration of sugars in the sap of the central vacuole attain their maximal values (38—45 days old ovules). At this age of ovules and in still younger ones one may observe in the sap of the central vacuole a drop of concentration of the undetermined compounds which doubtlessly take part in the synthesis of compounds of high molecular value and of low osmotic activity (proteins). It results out of the data given in Fig. 4 that sugars are the main element present in the sap of the central vacuole during the growth and development of the ovules. The results concerning the changes of sugar concentration in the central vacuolar sap in developing ovules are in agreement with the results of studies on the osmotic value and with the author's hypothesis (1960a, c).

The results of our studies on the physical and chemical properties

of the sap of the central vacuole (R y c z k o w s k i 1960a, b, c, d, 1961) suggest the following conclusions:

1. Changes of the osmotic value, viscosity and specific gravity of the sap of the central vacuole, as well as changes of the concentration of sugars and undetermined substances present in it proceed with great dynamism.

2. Absolute values of the above mentioned properties differ greatly in various plant species. In some cases, even, they are different for individual plant specimens of the same species (R y c z k o w s k i 1960a, c).

3. The elaboration of a universal nutrient medium for cultures in vitro, both of the endosperm and probably of the embryo in their early stages of development seems most unlikely.

4. It is also probable that nutrient media of different composition and concentration will have to be used during the development of the endosperm and the embryo.

SUMMARY

1. A study was performed on the changes occuring in the concentration of sugars in the sap of the central vacuole during the development of the ovules of three species (*Clivia* sp., *Haemanthus Katharinae, Haemanthus albiflos*). The method of S a m o g y i (1945 modified by Willis and Yemm (1955) was applied for the determinations of reducing and non reducing sugars. The accuracy of this method for dilutions of glucose and saccharose in the range of concentration from 12.5 to 100 µg per mililiter was respectively ± 1 and $\pm 2 \mu g$.

2. It was established that the concentration of all soluble sugars, reducing and non reducing, present in the sap of the central vacuole, changes in a characteristic way (Fig. 2 and 3) during the development and growth of the ovule. The reducing sugars are transformed into non reducing sugars which in turn most probably change into starch.

3. It was found that there is a dependence between the concentration of sugars in the central vacuolar sap, the amount of water in the ovule and its dry weight.

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STRESZCZENIE

1. W badaniach nad stężeniem cukrów w soku centralnej wakuoli zastosowano metodę Somogyi (1945), zmodyfikowaną przez Willisa i Yemma (1955). Dokładność metody dla roztworów glukozy i sacharozy w zakresie stężeń od 12,5 do 100 µg wynosiła ± 1, bądź też ± 1—2 µg.

2. Stwierdzono, że stężenie wszystkich cukrów rozpuszczonych w soku centralnej wakuoli, cukrów redukujących i cukrów nie redukujących zmienia się w trakcie wzrostu i rozwoju zalążków i że z cukrów redukujących odbywa się synteza cukrów nie redukujących, a z tych ostatnich najprawdopodobniej skrobia.

3. Ustalono, że istnieje pewien związek między stężeniem cukrów w soku centralnej wakuoli a ilością wody w zalążku oraz jego suchą masą.

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