# AN OSMOTIC MUTANT OF *ARABIDOPSIS THALIANA*

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*Summary*

A single recessive mutation in *Arabidopsis thaliana* (L.) Heynh. causes, at 23°C, distorted growth, narrower leaves, poor secondary root development, and complete sterility. Mutant plants grown at 18°C are very small with little differentiation and soon die, but at 28°C development is normal. The aberrant phenotype of the mutant is a consequence of its abnormally low cellular osmotic pressure, for, in aseptic culture at 23°C, additions to the medium of glucose, sucrose, or potassium sulphate, in amounts giving a substrate osmotic pressure greater than 2·5 atm, restore normal growth. Phenocopies induced by depressing the effective osmotic pressure of wild-type plants by growing them on mannitol closely resemble mutant plants in most respects. Glucose and sucrose both increase leaf width, but while the former substance increases only cell division, the latter's action is primarily to increase cell expansion. An incompatibility system is a secondary consequence of the mutation, for mutant pollen fails to grow on mutant stigmas, but will grow on wild-type stigmas; the reverse pollination is ineffective. Fertility in the mutant is restored by raising the substrate osmotic pressure, the relationship between osmotic pressure of medium as given by glucose and the number of seeds set per plant being linear. No differences are apparent in the carbohydrate metabolism or cell permeability of the mutant, and no cause could be found for its low osmotic pressure.

## I. INTRODUCTION

One of the major aims in physiological genetics is the tracing of the reaction chains which link the presence of a gene with a morphological phenotype. The general method of approach is ideally that of working back through decreasing levels of phenotypic complexity—morphological, anatomical, physiological, and eventually to the biochemical steps governed by single genes. This approach has been valuable in showing how genic interference in a basic reaction of development may, by upsetting the balance between subsequent morphogenic processes, give rise to very complex phenotypes. In practice, however, the analysis often stops at some level in the development of the phenotype that may be far removed from the primary developmental action of the gene. Usually, it is not possible to find the physiological difference that causes an anatomical change, probably because of the lack of knowledge of specific chemical activators in differentiation. This situation is particularly evident in the flowering plants, where mutant phenotypes seldom receive more than a general morphological or, at best, a histological description. The studies reported in this paper represent an attempt to provide a more detailed explanation of the action of a gene in controlling morphogenesis.

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## II. Material and Methods

The mutant under study (designated 1023/13) was first isolated from $X_2$ plants of *Arabidopsis thaliana* (L.) Heynh. growing on minimal agar medium containing 2 per cent. sucrose. It appeared as a plant smaller than the wild type, slower in flowering, and with pointed leaves. When grown on minimal medium lacking sucrose, mutant plants produced small, narrow, twisted, asymmetrical leaves, multiple short flower stalks, and failed to form seed. In the $X_2$ generation the ratio of wild type to mutant was 43 : 6, closely conforming to the expected 7 : 1 ratio for a mutation arising in one cell of a two-celled apical meristem in the irradiated seed. The $F_2$ segregation ratio of 57 : 14 ($\chi^2$ for 3 : 1 = 1·06, $P = 0{\cdot}4$–$0{\cdot}3$) indicates that the mutant phenotype results from a single gene change. No back mutations to wild type appeared in over 700 plants studied.

Mutant plants do not survive to flowering when grown in soil. Initial growth in soil is usually quite good, plants having normal-shaped leaves one-half to two-thirds the size of the wild type. However, after the first two or three pairs of leaves, differentiation becomes abnormal and plants soon die.

The mutant was examined on plants grown aseptically on chemically defined media under conditions described by Langridge (1957, 1958). Most growth experiments were made with substrates with the tonicity altered by potassium sulphate glucose, sucrose, or mannitol. The glucose and potassium sulphate used were of "analytical reagent" grade, while sucrose solutions were purified by boiling with activated charcoal for 5 min. Commercial mannitol was quite toxic to the plants unless it was first purified by extraction with ether at pH 3 and then at pH 8 and recrystallized.

## III. Results

During the process of isolation and initial testing it was noted that, although the mutant showed extreme disorganization of growth when grown on minimal medium, its phenotype when grown on sucrose-containing substrate was almost that of the wild type. Growth was not improved by supplements such as coconut milk, amino acids, vitamins, yeast extract, and nucleic acid hydrolysate.

### (*a*) *The Effect of Altering the Osmotic Pressure of the Substrate*

As the preliminary tests indicated that the mutation had induced a requirement for sucrose, experiments were conducted to distinguish between the nutritional and osmotic effects of the sugar. For this purpose, mutant plants were grown on minimal agar plus sucrose, glucose, potassium sulphate, or mannitol, each added in amounts calculated to give substrates with an osmotic pressure of 2 atm. The osmotic pressure of the minimal medium was approximately 0·5 atm.

(i) *Penetrance and Expressivity of the Mutant Genotype.*—As may be seen from Plate 1, which illustrates mutant plants (26 days old) on different concentrations of sucrose, there may be a complete gradation in phenotype from a very small lethal form, to one which closely approaches that of the wild type. There is also variation in phenotypic expression (expressivity) with any treatment which, in general, tends to favour the growth of the mutant. The number of homozygous

recessive individuals which exhibit an abnormal phenotype with a given treatment represents the penetrance of the mutant gene under these conditions. As may be seen from Table 1, the expressivity of the gene varies with the treatment and increases as growth proceeds, while Figures 1 and 2 show that its penetrance decreases as the osmotic pressure of the medium increases up to 2·5 atm.

TABLE 1

EFFECT OF OSMOTIC AGENTS ON THE EXPRESSION OF THE MUTANT PHENOTYPE

Temperature 23°C

| Osmotic Agent | Osmotic Pressure of Substrate (atm) | No. of Plants | Age of Plants (days) | Phenotype | | |
|---|---|---|---|---|---|---|
| | | | | Normal (%) | Partly Abnormal (%) | Abnormal (%) |
| Nil | 0·5 | 17 | 16 | 0 | 18 | 82 |
| | | | 25 | 0 | 12 | 88 |
| | | | 29 | 0 | 0 | 100 |
| Sucrose (100 mg/plant) | 2·0 | 18 | 16 | 88 | 6 | 6 |
| | | | 25 | 67 | 22 | 11 |
| | | | 29 | 56 | 22 | 22 |
| Glucose (55 mg/plant) | 2·0 | 20 | 16 | 75 | 20 | 5 |
| | | | 25 | 60 | 5 | 35 |
| | | | 29 | 50 | 10 | 40 |
| Potassium sulphate (21·75 mg/plant) | 2·0 | 13 | 16 | 70 | 15 | 15 |
| | | | 25 | 31 | 7 | 62 |
| | | | 29 | 7 | 23 | 70 |
| Mannitol (55 mg/plant) | 2·0 | 16 | 16 | 0 | 0 | 100 |

The differences in expressivity, and hence of penetrance, are due firstly to slight but uncontrollable inequalities in the environment, and secondly to the differential occurrence of delayed expression of the mutant phenotype during growth. The effect of the mutation is expressed primarily on leaf growth and with most treatments a proportion of the plants may have normal formation of the first or second leaf pair while subsequent leaves become abnormal. Such reversion to the mutant phenotype suggests that the plant's requirement for a component of the substrate may exceed its rate of uptake. The frequency of this reversion is very much greater with potassium sulphate than with glucose in the substrate (Figs. 1 and 2).

The fact that potassium sulphate is almost as effective as glucose or sucrose in restoring the wild-type phenotype, at least initially, indicates that the mutant requires sugar because of its osmotic properties, and that the mutation has affected

the osmotic relations of the plant. Mannitol, on the other hand, induces full penetrance and eventually causes the death of mutant plants. At the concentration shown in Table 1, mannitol has no pronounced effect on wild-type plants.

Plant cells have repeatedly been found to be impermeable to mannitol (Collander and Bärlund 1933; Thimann, Slater, and Christiansen 1950), so the mutant cannot require an increased osmotic pressure in the substrate. On the basis of the data on phenotype expression it appears possible that the mutant needs a source of solutes with which it may increase the internal osmotic pressure.

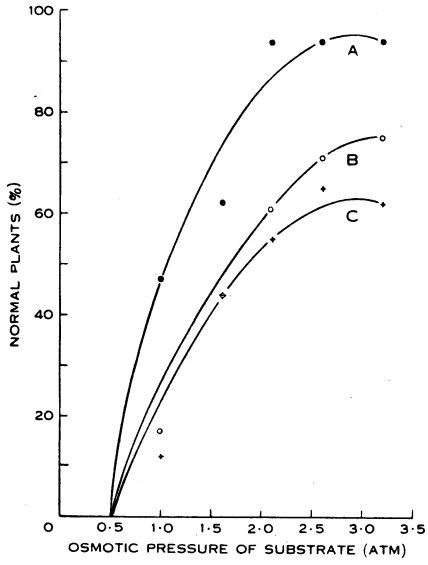



Fig. 1

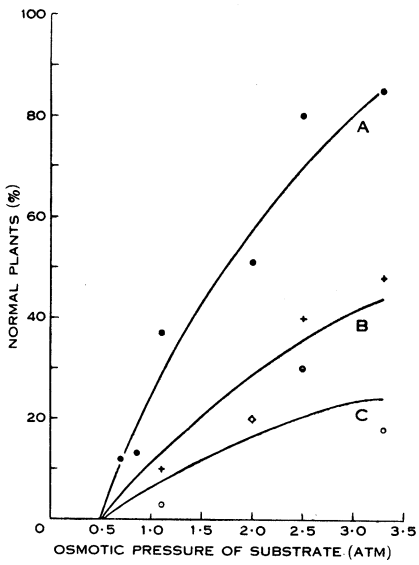



Fig. 2

Fig. 1.—Percentage of phenotypically normal plants obtained from plants of mutant genotype by increasing the osmotic pressure of the substrate by the addition of glucose. *A*, plants 14 days old; *B*, 18 days old; *C*, 22 days old.

Fig. 2.—The same as Figure 1, but with potassium sulphate as the osmotic agent.

(ii) *Leaf and Root Growth.*—As already mentioned, one of the most pronounced effects of the mutation is to cause the leaves to be very narrow, rather short, and more or less closely curled. However, as the concentration of glucose or potassium sulphate in the medium increases, the leaves become wider and longer. The leaf curling prevented the accurate measurement of leaf lengths, but the width of the lamina of the first leaf was taken as an index of the effect of treatments upon leaf growth (Fig. 3). Leaf size and shape are closely related to normality of phenotype and, as expected, glucose is most effective in increasing leaf width, with potassium sulphate being slightly inferior. The minimum concentration of glucose that gives maximum leaf width is about 80 mg per plant corresponding to a substrate osmotic pressure of 2·6 atm. Mannitol, even at low concentration, markedly reduces the leaf width of the mutant, further confirming the inferences drawn in the previous section. The leaf width of the wild type is $4{\cdot}0 \pm 0{\cdot}25$ mm, and with equimolar

concentrations of glucose $3\cdot8\pm0\cdot17$, sucrose $3\cdot9\pm0\cdot17$, and potassium sulphate $4\cdot6\pm0\cdot14$ mm.

Primary root growth is not affected by the mutation but the number of secondary roots is halved (Table 2), and this in turn may retard water absorption.

TABLE 2

EFFECT OF OSMOTIC AGENTS ON ROOT GROWTH

Age of plant 17 days, temperature 23°C

| Osmotic Agent | Osmotic Pressure of Substrate (atm) | Mutant | | Wild Type | |
|---|---|---|---|---|---|
| | | Primary Root Length (mm) | Number of Secondary Roots | Primary Root Length (mm) | Number of Secondary Roots |
| Nil | 0·5 | $24\cdot8\pm1\cdot0$ | $9\cdot5\pm1\cdot0$ | $29\cdot0\pm0\cdot3$ | $18\cdot4\pm0\cdot5$ |
| Glucose (55 mg/plant) | 2·0 | $26\cdot9\pm0\cdot2$ | $15\cdot3\pm0\cdot5$ | $28\cdot5\pm0\cdot7$ | $18\cdot2\pm0\cdot8$ |
| Potassium sulphate (21·75 mg/plant) | 2·0 | $21\cdot2\pm0\cdot8$ | $14\cdot4\pm0\cdot8$ | $28\cdot0\pm0\cdot5$ | $17\cdot8\pm0\cdot6$ |
| Mannitol (55 mg/plant) | 2·0 | $27\cdot5\pm0\cdot6$ | $4\cdot9\pm1\cdot0$ | $26\cdot4\pm0\cdot6$ | $15\cdot4\pm0\cdot6$ |

The poor secondary root growth evidently results from some effect of osmotic insufficiency, for potassium sulphate is as effective as glucose in increasing root number.

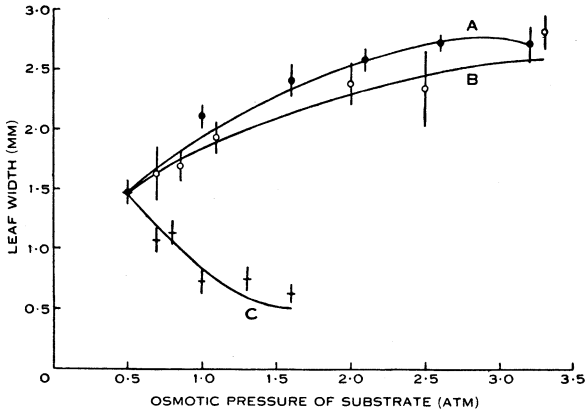



Fig. 3.—Widths of the laminae of mutant plants with increasing osmotic pressure of the medium. *A*, potassium sulphate; *B*, glucose; *C*, mannitol.

(iii) *Fertility.*—When grown on minimal medium the mutant forms numerous short pods which are completely devoid of seed. However, there is a linear relationship between the number of seeds set per plant and the osmotic pressure of the

substrate (as given by glucose) (Fig. 4). Potassium sulphate at low concentrations permits the formation of a few seeds, but at higher concentrations it causes the flower stalks to die back at the time of flowering. Even in the wild type, potassium sulphate, while allowing good vegetative growth, causes similar dying of the flower stalks and almost complete sterility (Table 3). Mannitol kills mutant plants before flowering and results in nearly complete sterilization of the wild type. Sucrose is not as effective as glucose in restoring fertility in the mutant and it is slightly inhibitory in the wild type.

Pollinated stigmas of mutant plants growing on minimal medium were squashed, stained with cotton blue in lactophenol, and examined microscopically. In contrast with the situation in wild-type plants, mutant pollen usually failed to germinate on

TABLE 3

FERTILITY OF MUTANT AND WILD TYPE GROWN ON DIFFERENT SUBSTRATES

| Osmotic Agent | Substrate Osmotic Pressure (atm) | Temperature (°C) | No. of Seeds per Plant | |
|---|---|---|---|---|
| | | | Mutant | Wild Type |
| Nil | 0·5 | 23 | 0 | 275±32 |
| | | 28 | 0 | 0 |
| Glucose (55 mg/plant) | 2·0 | 23 | 136±8 | 265±21 |
| | | 28 | 0 | 44±12 |
| Sucrose (100 mg/plant) | 2·0 | 23 | 89±11 | 266±13 |
| Potassium sulphate (21·75 mg/plant) | 2·0 | 23 | 0 | 0·8±0·6 |
| Mannitol (55 mg/plant) | 2·0 | 23 | 0 | 9±3 |

the mutant stigma or the pollen tubes grew only a short distance down the style. However, pollen from mutant plants germinates and effects fertilization on wild-type stigmas, although wild-type pollen does not germinate on mutant stigmas. A secondary effect of this mutation, therefore, is the establishment of an incompatibility system in which unilateral out-crossing is necessary for survival of the mutant gene.

### (*b*) *Effect of Temperature*

The mutant shows a pronounced change in phenotype with change in temperature. Plants in all the above experiments were grown at a uniform temperature of 23°C, in which the mutant phenotype is well expressed and viability reasonably good. At 18°C mutant plants are extremely small and die after about 14 days growth, but at 28°C differentiation is always normal although the leaves are smaller than in the wild type. Also, while wild-type plants have decreased growth as shown by dry weight at 28°C, the dry weight of the mutant is three times greater at 28 than at 23°C (Table 4). Similarly, the leaf width of the mutant growing on minimal

medium is twice as great at the higher temperature, but that of the wild type is significantly decreased (Table 5).

The high temperature (28°C) does not restore the fertility of mutant plants but even the wild type is completely sterile at this temperature (Table 3). Fertility in the wild type is partially restored by glucose, but sugars have no such effect on seed formation in mutant plants.

TABLE 4

DRY WEIGHTS OF MUTANT AND WILD TYPE

| Temperature (°C) | Mutant | | Wild Type | |
|---|---|---|---|---|
| | Number of Plants | Dry Weight (mg) | Number of Plants | Dry Weight (mg) |
| 23 | 14 | 0·9±0·01 | 12 | 5·6±0·01 |
| 28 | 15 | 2·8±0·1 | 11 | 3·2±0·07 |

### (c) *Phenocopy Production*

The action of glucose and potassium sulphate in restoring growth suggests that this mutation causes a low solute concentration in the cells and thus a low osmotic pressure. Attempts were made to measure directly the osmotic pressure of wild-type and mutant plants. The plasmolytic method of measuring osmotic pressure

TABLE 5

LEAF WIDTHS OF MUTANT AND WILD TYPE

| | Treatment | Leaf Width (mm) | |
|---|---|---|---|
| | | 23°C | 28°C |
| Mutant | Nil | 1·1±0·12 | 2·1±0·12 |
| | Glucose (55 mg/plant) | 1·9±0·1 | 2·8±0·08 |
| Wild type | Nil | 4·0±0·26 | 3·0±0·11 |
| | Glucose (55 mg/plant) | 3·8±0·12 | 3·2±0·08 |

is feasible only with especially favourable plant tissues, while insufficient cell sap was obtainable for cryoscopic determinations, even on a microscale. The mechanical vibration method of Virgin (1955) for the estimation of turgor pressure also proved

unsuitable. However, a very approximate estimate of osmotic pressure was obtained by observing the curvature of split petioles in a graded series of sucrose solutions. This method gave an osmotic pressure of 6·5 atm for wild-type tissue and 5·0 atm for mutant tissue, both grown on minimal medium.

As a test of the correctness or otherwise of the inference that the characteristic phenotype of the mutant resulted from an abnormally low internal osmotic pressure, an attempt was made to induce a phenocopy from the wild type. One consequence of a low osmotic pressure in the vacuolar sap is a reduced turgor pressure which is

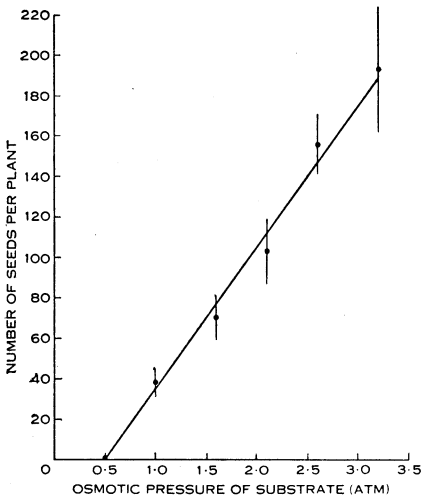



Fig. 4

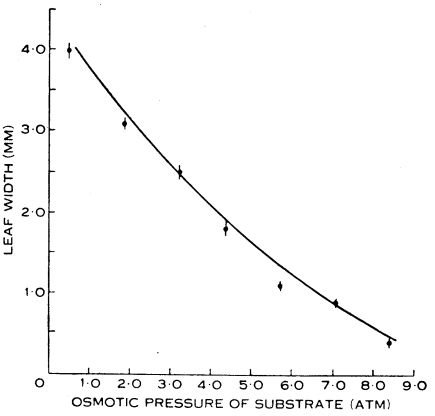



Fig. 5

Fig. 4.—Number of seeds set by mutant plants as the osmotic pressure of the medium is increased by the addition of glucose.

Fig. 5.—Widths of the laminae of phenocopies of the mutant. Wild-type plants grown on medium containing mannitol.

equal to the difference between the osmotic pressure of the cell contents and that of the surrounding medium. Thus the internal turgor pressure (or effective osmotic pressure) of a plant may be decreased by increasing the osmotic pressure of the substrate with a substance such as mannitol to which the cell membrane is impermeable. Thus, for the production of a phenocopy of the mutant under study, wild-type plants were grown at 23°C on agar containing graded concentrations of purified mannitol.

The effect of the mannitol, even at low concentrations, was to change the same characters as the mutation affects, i.e. leaf width, cell growth, and fertility. The phenotype which most closely approached that of the mutant (at 23°C on minimal medium) was found in plants growing on 150 mg of mannitol per test tube, corresponding to an osmotic pressure of 4·0 atm.

Figure 5 shows that the leaf width of the wild type decreases markedly as the concentration of mannitol in the substrate increases, and therefore, as the

consequent turgor pressure of the cells decreases. The turgor pressure of the wild type must be reduced by 4·5 atm before its leaf width corresponds to that of the unsupplemented mutant.

### (*d*) *Cell Growth*

Following a description of the expression of the mutation on plant habit and tissue growth, the next logical step was an examination of cell growth. The first leaves, when mature, were fixed in Navashin's fluid, embedded in paraffin, sectioned transversely at 20 $\mu$, and stained in saffranin and fast green.

TABLE 6

EFFECT OF SUPPLEMENTS AND TEMPERATURE ON LEAF AND CELL GROWTH

Measurements and cell counts made to the midrib on the wider half of the leaf to avoid differences resulting from bilateral asymmetry. Mean cell diameter obtained by dividing the half-leaf width by the number of cells

| Treatment | Growing Temperature (°C) | Mutant | | | Wild Type | | |
|---|---|---|---|---|---|---|---|
| | | Mean Cell Diameter ($\mu$) | Number of Cells | Leaf Width to Midrib (mm) | Mean Cell Diameter ($\mu$) | Number of Cells | Leaf Width to Midrib (mm) |
| Nil | 23 | 14·8 | 25 | 0·37 | 24·1 | 101 | 2·41 |
| Nil | 28 | 27·3 | 42 | 1·15 | 14·8 | 100 | 1·48 |
| Glucose (55 mg/plant) | 23 | 14·9 | 91 | 1·36 | 23·4 | 119 | 2·79 |
| Potassium sulphate (21·75 mg/plant) | 23 | 23·2 | 65 | 1·51 | 28·5 | 112 | 3·19 |

As the leaves of the mutant often tend to be bilaterally asymmetrical, leaf width was measured only to the midrib, and the number of adjacent palisade cells in this cross section of the leaf counted. Mean cell width was obtained by dividing the number of cells into the half-leaf width.

The results of measurements of cell number, cell size, and leaf width for mutant and wild type plants grown on different substrates and at 23 and 28°C are presented in Table 6. A comparison of the numbers and sizes of the cells in the mutant and wild type on minimal medium shows that the narrow leaf width of mutant plants is caused by its having few cells which are much smaller than normal. The addition of glucose to the substrate has a very marked effect in increasing cell division, although it causes no increase in cell size. Potassium sulphate, on the other hand, results in much larger cells even in the wild type, but cell number is not so greatly increased. The different modes of action of these two substances in increasing leaf width is

more clearly seen in Figures 6 and 7. When glucose is supplied, the graph for cell number closely follows that for leaf width; but with potassium sulphate, it is the graph for cell size which approximates to the leaf-width graph.

The morphological effect of an increase in temperature is brought about through a doubling of cell size with a lesser stimulation of cell division. The expansion of wild-type cells, however, is markedly reduced. As an increase in temperature normally causes a decrease in the osmotic pressure of plant cells (Went 1955), it is assumed that the higher temperature repairs the basic biochemical deficiency in the mutant.

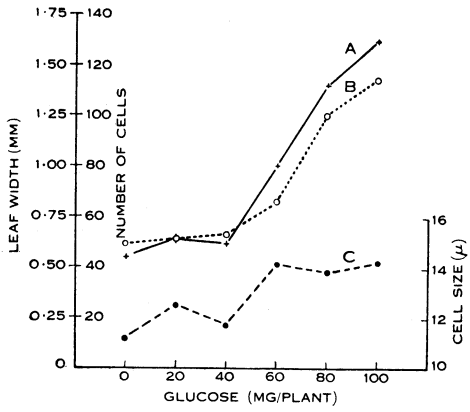



Fig. 6

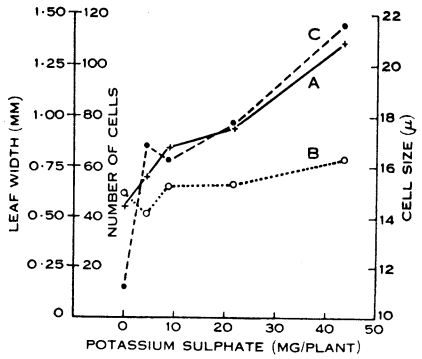



Fig. 7

Fig. 6.—Leaf widths to the midrib, and the number and size of the cells contained therein, of mutant plants growing on increasing concentrations of glucose. *A*, leaf width; *B*, number of cells; *C*, cell size.

Fig. 7.—The same as Figure 6, but with potassium sulphate added to the medium.

The fact that the mutation affects both the number and size of leaf cells may seem at variance with the hypothesis that its phenotypic expression results solely from decreased osmotic pressure. However, this hypothesis has been verified by an examination of the cell behaviour of leaves of the phenocopies (wild-type plants on mannitol substrates). Here the action of mannitol, which can only be due to a lowering of internal osmotic and turgor pressure, is to decrease cell number and size as does the mutation (Fig. 8).

### (*e*) *Carbohydrate Metabolism and Cell Permeability*

Attempts were made to find the cause of the low osmotic pressure of cells of mutant plants. Two possibilities seemed worth investigating. Firstly, that there is a deficiency in carbohydrates causing a low cell solute concentration and a lack of energy available for cell division. Secondly, that an increased permeability of mutant cells leads to a rapid loss from the plant of both sugars and inorganic constituents.

(i) *Carbohydrate Metabolism.*—Manometric measurements of photosynthesis and respiration of whole plants using the $CO_2$ buffer system described by Pardee (1946) revealed no difference between normal and wild type.

The formation and dissolution of starch in the leaf cells was next examined. Leaf tissue was sectioned at 50 $\mu$ and stained in a solution of iodine plus potassium iodide. The chloroplasts of both mutant and wild-type plants contained abundant starch both at 23 and 28°C. No discernible difference was found in the rate of

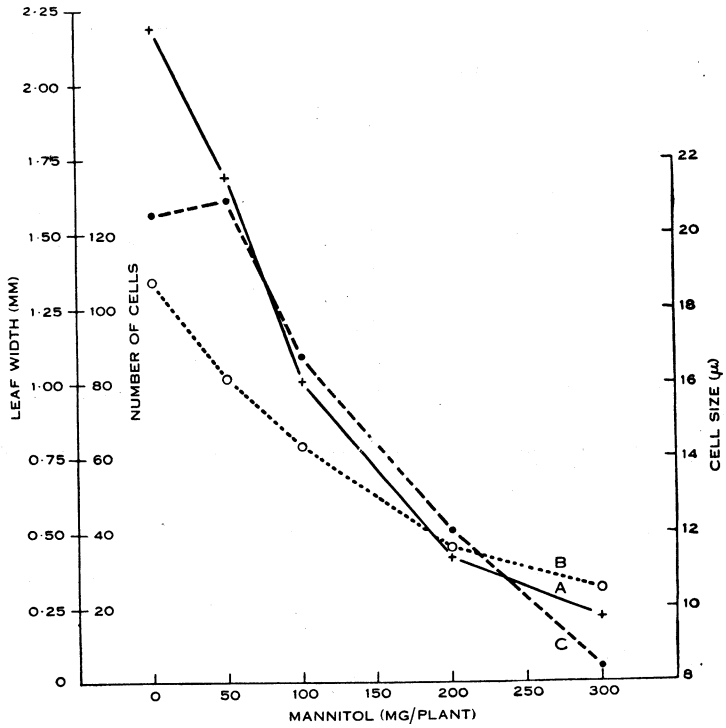


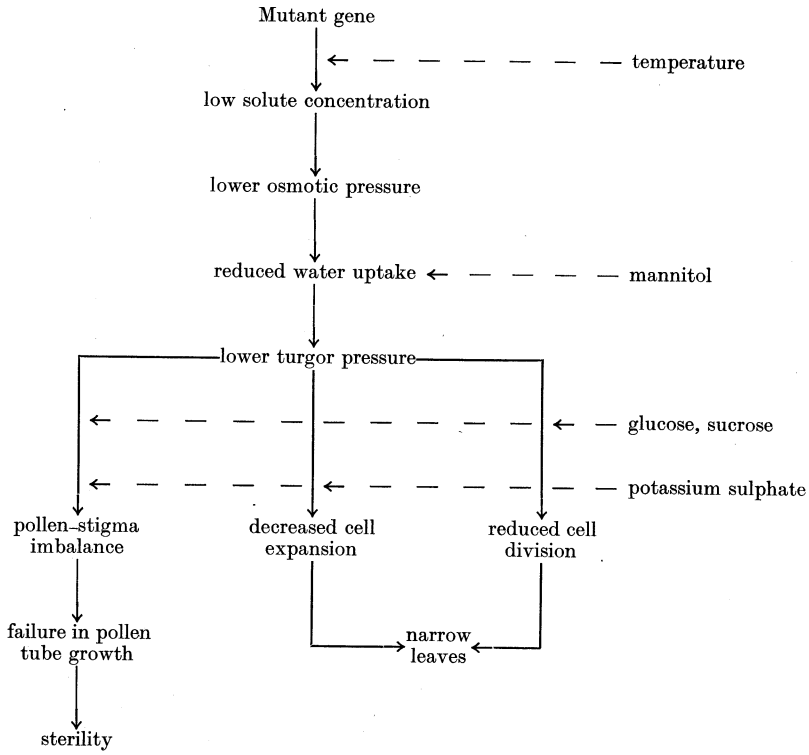

Fig. 8.—Leaf widths to the midrib, and the number and size of the cells contained therein, of wild-type plants growing on increasing concentrations of mannitol. *A*, leaf width; *B*, number of cells; *C*, cell size.

breakdown of the starch in the dark at any of the temperatures tested. An examination of the ability of mutant and wild-type leaf tissue to synthesize starch from glucose 1-phosphate was made following the method of Badenhuizen (1955). Again no consistent differences were found.

(ii) *Cell Permeability.*—For the study of permeability, the relatively large semi-transparent cells forming the stigma surface were used. Permeability to water was measured by plotting the number of cells plasmolysed against time when stigmas were immersed in a 1M solution of glucose. Further tests of permeability were made from rates of deplasmolysis of fully plasmolysed cells in 1M solutions of urea or potassium sulphate. In all experiments the behaviour of mutant cells fully conformed with that of wild-type cells.

## IV. Discussion

From the data obtained in the above experiments, the interrelated consequences of this mutation may be summarized diagrammatically. Some of the steps, difficult to measure quantitatively in this material, have been inferred from previous work, while the order of other steps has been deduced from the pattern of effect produced by supplements or temperature.




The study of this mutant is incomplete because no cause could be found for the low osmotic pressure of mutant cells. There could be at least four possible reasons for this reduced osmotic pressure:

(1) A lowered rate of metabolism or an alteration in balance between photosynthesis and respiration resulting in a lower solute level in the cell vacuoles, or insufficient energy for osmotic work.

(2) A block in starch degradation which would lock up the products of photosynthesis in an osmotically inactive form.

(3) An increase in cell membrane permeability reducing osmotic pressure through excessive leakage of solutes from the root cells.

(4) Alterations in those physical properties of the cytoplasm which are responsible for imbibition or ion retention by electrostatic and other forces.

It has not been possible to test all of these possibilities, although the first three appear to be eliminated as primary effects of the mutation by the experiments described in Section III (*e*).

The finding that both cell size and cell number were decreased in the mutant was an unexpected one, for general opinion (e.g. Burström 1951) is that osmotic pressure, acting through turgor pressure, is merely a driving force in cell expansion. Nevertheless, the reduction in the number of cells in wild-type plants grown on mannitol substrates proves that osmotic pressure, or the hydrostatic forces it affects, may also be a regulating factor in cell division.

Some explanation is needed for the different activities of glucose and potassium sulphate in increasing cell growth. If the osmotic deficiency of mutant cells resulted in a lack of energy source through exosmosis, this could be repaired by the absorption of glucose from the substrate. This rate of absorption (about 2 mg per day in plants of maximum vegetative growth) may, however, be insufficient to maintain an increased osmotic pressure. Glucose, therefore, could lead to an increase in cell division without affecting cell expansion, while potassium sulphate, nutritionally inert and more easily absorbed, would favour cell expansion. This explanation is unsatisfactory in that no account is taken of the fact that both substances are effective in restoring the fertility of the mutant.

This system of self-incompatibility but non-reciprocal cross-compatibility with wild type is partly comparable with the naturally occurring incompatibility mechanism of *Linum grandiflorum* (Lewis 1943). Both have as a causal basis the presence of unsuitable osmotic pressure differences between pollen and style. However, the disparity in osmotic pressure does not seem to be the full explanation for the incompatibility system of *Linum*, and it may not be the sole cause of sterility in the *Arabidopsis* mutant.

In its apparent deficiency in water relations, this mutant resembles the morphological mutant "cut" in *Neurospora crassa* (Kuwana 1953). This *Neurospora* mutant required, for normal hyphal growth, a relative humidity in excess of 94 per cent. It also was sensitive to the osmotic pressure of the medium, but, unlike the *Arabidopsis* mutant, it gave a decreased rate of growth as compared with the wild type when the osmotic pressure was raised.

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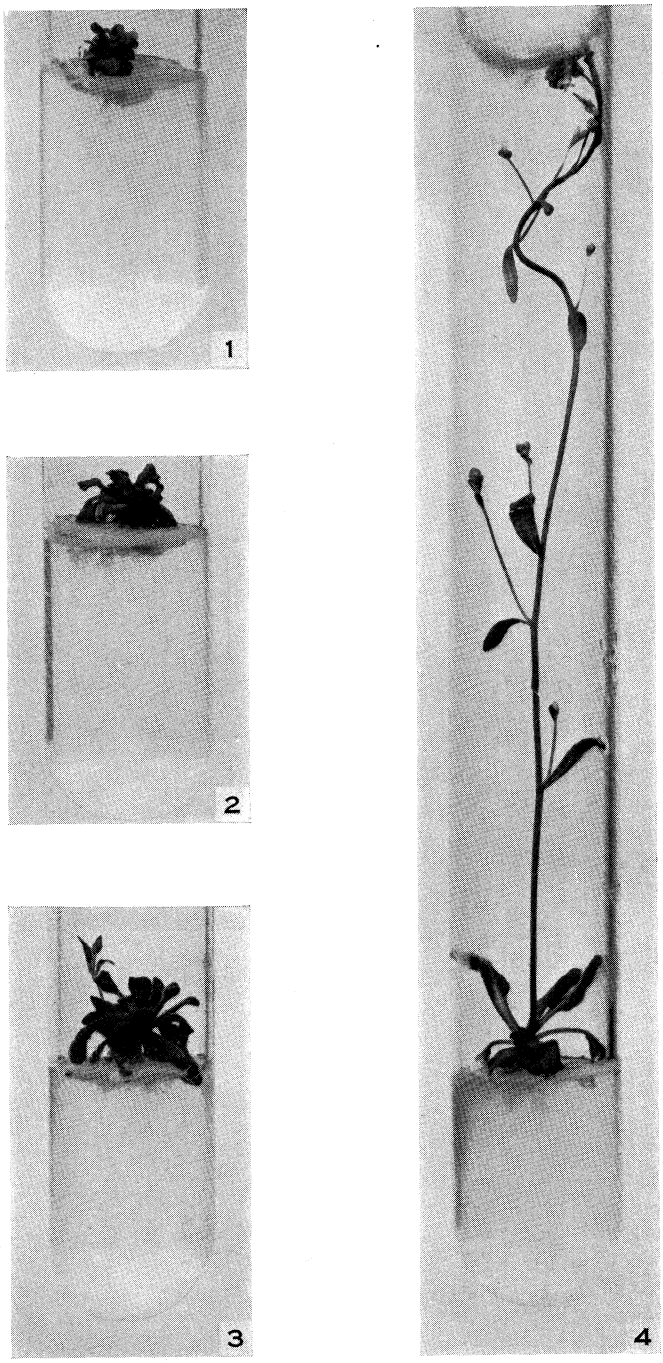
AN OSMOTIC MUTANT OF ARABIDOPSIS THALIANA




Mutant plants, 26 days old, growing on minimal medium with and without added sucrose. *1*, no sucrose; *2*, 20 mg sucrose; *3*, 50 mg sucrose; *4*, 100 mg sucrose.