Stomata and Transpiration of Droopy Potatoes
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Summary. A diploid potato (Solanum tuberosum) mutant called droopy wilts
easily. Excised leaves of the mutant lost weight, and hence water, more rapidly
and had many more open stomata than leaves of a normal sibling. Further, the
stomata of abnormal plants remained open in wilted leaves. When the stomata of
the abnormal mutant were closed by a chemical spray, its excised leaves lost water
no more rapidly than normal. Thus, the wilting of the mutant must be caused by
wide stomata. The wilting of the abnormal leaves and the small dry weight of the
plants indicate the advantage of the stomatal hydrostat in the normal plants.

The recessive gene droopy (dr) in potatoes is
expressed as tall, slim plants whose leaves wilt
during the day (4). This wilting results in much
tip scorching and premature leaf fall. Since re-
ciprocal grafting experiments showed that the
droopy phenotype was not due to a defect of the
root or vascular system (3), we investigated the
leaf to discover the inherited aberration that caused
the wilt.

Materials and Methods

The mutant droopy (dr) is one of many deleteri-
ous recessives known in the cultivated diploid po-
tatoes (Solanum tuberosum) of the Andes (3). All
such diploids (with 2n = 24) are outbreeders that
have an oppositional-allele, (S) incompatibility sys-
tem. Geographically, they are distributed down the
Andes from Venezuela to Bolivia. The stocks from
which the gene was isolated came from Colombia
(4). In the glasshouse in summer in England,
plants that express the deleterious character are
generally viable and fertile, though tall, slim and
weak in appearance; in this they contrast with most
other potato mutants, which are commonly very
weak indeed or even lethal. Though dr is genetic-
ally a simple recessive, it segregated first in a highly
aberrant ratio which was found to be due to linkage
with the S-(incompatibility-) locus (4). The ma-
terial used in the present study was a backcross
family of the type Dr/dr ♀ × dr/dr ♂ in which the
expected 1:1 ratio is affected by S-linkage.
Equal numbers of normal and droopy siblings were
therefore available for comparison.

The diploid potatoes being outbreeders, there is
always considerable variation in mutant expres-
sion. For this study, 12 vigorous normal plants
were compared with 12 of the more extremely ex-
pressed droopy plants, all taken from one family
of 38 plants that segregated the expected ratio
(18 normal:20 droopy).

During March and April, the seed was planted
in soil in a diffusely illuminated room. In early
June, all seedlings were transplanted to a green-
house where refrigeration maintained a temperature
of about 20° and a humidifier maintained a relative
humidity of 80 to 100 %. The moist atmosphere
permitted the plants that expressed dr to live, but
both transpiration and wilting still occurred. By
August, the normal plants had grown to a dry weight
of about 10 g and a height of about 25 cm. The
12 plants at each extreme of expression were then
selected for experiment, as described above.

Since the grafting experiments (3) had indi-
cated that the aberration resided in the leaves,
we determined the evaporation from excised leaves.
One-half minute after excision from the plant, the
leaves were weighed. They were then exposed to
sun and wind and weighed again at 1, 2 and 4
minutes. Abnormal and normal leaves were alter-
ned to eliminate effect of atmosphere from com-
parison. During this evaporation, solar radiation
was greater than 0.4 cal cm⁻² min⁻¹ and wind was
180 to 290 cm sec⁻¹ at a nearby observatory. The
transpiration can be expressed as mg loss per cm².
Since leaf weight was related to area in the same
manner in both kinds of plants, however, the same
conclusion was reached whether transpiration was
expressed as mg cm⁻² min⁻¹ or percentage of the
weight at 0.5 minute lost per minute. The per-
centage method was employed, and hereafter per-
centage weight and percentage loss of weight are
denoted by the single words weight and loss. The
losses were subjected to analysis of variance, and
the significance of differences is indicated by
P = 0.01 or P = 0.05.
Stomatal area was measured by microscopic examination of replicas of the epidermis (5). Replicas in any 1 investigation were taken within 10 minutes. Solar radiation was always greater than 0.3 cal cm\(^{-2}\) min\(^{-1}\) during replication. The area was calculated by multiplying the number of open stomata per cm\(^2\) by the modal size of the open stomata. Alternatively, the stomata wider than 1 micron were classified as open, and the percentage open was estimated.

Phenylmercuric acetate narrows the stomata and accordingly decreases transpiration from excised tobacco leaves (6). Therefore, when it was desired to close stomata, 1 mM phenylmercuric acetate and 0.1% (v/v) wetting agent (Triton X100, Rohm and Haas, Phila.) in water was sprayed on individual leaves until they were thoroughly wet. The leaves were observed after they had dried.

**Results and Discussion**

When leaves were excised from plants, those from *dr* plants lost water more rapidly than normal leaves (fig 1); indeed, the slowest loss from an abnormal was faster than the fastest from a normal leaf. A similar result was obtained in a second lot of 10 plants on a later day. In both experiments, the losses for 3.5 minutes after the first weighing were significantly (P = 0.01) faster in abnormal than in normal leaves.

In a second experiment, the leaves were permitted to dry for a longer time with the result shown in figure 2. The abnormal leaves promptly became visibly flaccid while the normal ones remained turgid for some time. Nevertheless, the abnormal continued to lose water more rapidly than normal ones despite wilting. The difference between rates of loss was significant (P = 0.01).

At this point, we concluded that all or a large portion of the cause of the wilting of the abnormal plants was rapid transpiration that continued even after the leaves had wilted. Because this suggested a stomatal defect, replicas of the lower epidermis of intact leaves of 12 abnormal and 12 normal plants were examined. Both classes of plants had about 500 stomata mm\(^{-2}\), and the stomata were 9 to 22 microns long. The opening of the stomata, however, was different. The median percentage of stomata open in the 12 abnormal plants was 50% with percentages in individual plants ranging from 0 to 96. In 11 of the normal plants, no stomata were found open; and in the remaining plant, only 12% were open.

Evaporation rates are related to stomatal area in the same individual in figure 3. Shortly after the evaporation from the leaves of figure 1 had been measured, replicas were made of other leaves in these 10 plants from which leaves had been excised. In 7 leaves, numerous stomata were visibly open, and the pore or stomatal areas per cm\(^2\) are plotted in figure 3; since these varied 200-fold, logarithms are employed. Where no stomatal opening could be seen, the area is called closed. The evaporation rate was expressed as the relative loss in weight between 0.5 and 4 minutes after excision. On another day and with other plants, results of figure 3 were essentially duplicated. Clearly, the
rapid evaporation associated with the wilting of the abnormal plants is in turn associated with wide stomatal pores.

Although the evidence of replicas has established the width of stomatal pores in the intact abnormal leaves, the stomatal condition that permitted continued rapid evaporation from excised \(dr\) leaves remained to be established. In a pair of leaves, the stomata of the normal one were all closed, i.e. narrower than 1 micron, before and after excision. In the abnormal one, 47% of the stomata were open wider than 1 micron before excision, and 50% were open even after 2.5 minutes of drying. On another day a normal leaf was excised, lost 4% of its weight in 3.5 minutes of drying, and had only 1 stoma in 66 open. During the same hour, 3 abnormal leaves lost 13, 14 and 11% of their weight in 3.5 minutes of drying and had 35, 86 and 70% of their stomata open. Thus, the stomata of plants expressing \(dr\) lack the ability to close as the leaf loses water and thus do not conserve leaf hydration as normal ones do. Since stomatal closure is caused by the loss of turgor of the guard cells (1), the foregoing observations suggest that the abnormality of stomata expressing \(dr\) is remaining relatively turgid during wilting.

Experimental proof that the open stomata were the entire cause of the rapid evaporation from abnormal leaves was still lacking although the 2 phenomena were correlated (fig 3). In a search for experimental proof that open stomata alone and not open stomata plus rapid cuticular evaporation was the cause, leaves were sprayed with phenylmercuric acetate, which causes stomata in many species to close. A total of 5 leaves was sprayed with phenylmercuric acetate on 3 plants on 3 occasions. On 1 occasion, the abnormal stomata were not wide and closure by the spray could not be detected microscopically in replicas of the leaves; this did not, of course, show that the stomata were not susceptible to phenylmercuric acetate. On the other occasions, however, the percentage of open stomata was decreased from 27 to 8 and from 75 to 1% by the spray of phenylmercuric acetate. If closure of the abnormal stomata is caused by a loss in turgor of the guard cells relative to subsidiary cells as is generally the case (1), phenylmercuric acetate must cause a migration of water from the guard cells or a permeability of guard cell membranes that general wilting does not.

The loss of water from the sprayed leaves after excision was compared with that from unsprayed leaves from the same abnormal plant and from a normal plant (fig 4). The evaporation from the unsprayed abnormal leaves was significantly \((P = 0.01)\) faster than from normal ones, as had been observed before. But after the spray, the abnormal leaves lost no more water than the normal ones and remained turgid as long as the normal ones. Since rapid evaporation from \(dr\) (fig 1,2) accompanies wide stomata (fig 3) and since experimental closure of abnormal stomata decreases evaporation rate to that of normal (fig 4), the genetic aberration caused by \(dr\) is evidently inability of stomata to close when leaf hydration decreases.

Although transpiration of water and not assimilation of carbon dioxide is the subject of this report, both gases do pass through the stomata. Thus, the assimilation of carbon dioxide in photosynthesis in a
well-hydrated tobacco leaf is greatest when stomata are widest (6). Dry weight, of course, indicates the net assimilation per plant. Therefore, the effect of \( dr \) upon the dry weight is presented in Table I as an indication of the net uptake of carbon dioxide through the stomata.

Five normal and 5 abnormal plants were harvested about 4 months after the seed had been sown. Abnormal plants weighed significantly \( (P = 0.01) \) less than normal plants (Table I). Further, the shoot weighed more than the root in the abnormal, and the relation was opposite in normal plants; this interaction was significant \( (P = 0.01) \). The relatively large shoot/root ratio may have exaggerated the difficulties of supplying adequate water to the rapidly transpiring, wide stomata of the abnormal leaf. Reciprocal grafting experiments showed, however, that the roots from \( dr \) plants provided adequate water for normal shoots (3). This result is more simply explained by the inference that \( dr \) roots generally function satisfactorily than by the inference that grafting on a normal shoot makes formerly faulty roots into functional ones. Accepting the first inference, we conclude that the large shoot/root ratio was not a major contributor to the expression of \( dr \).

The great difference in growth of the entire plant was not surprising for the abnormal plants expressing \( dr \) are always weaker than normal ones. In 2 earlier attempts to grow these potatoes in Connecticut, refrigeration and humidification had not been used, and the abnormal potatoes had died as young seedlings. Even in the mild, moist climate of England, they look poor though they survive quite well in the glasshouse.

Although wide stomata permitted rapid assimilation of carbon dioxide in a well-hydrated tobacco leaf (6), wide stomata that remained wide in wilted \( dr \) leaves accompanied small net assimilation of carbon dioxide per plant. Clearly, the great opportunity for carbon dioxide to enter the leaf through the wide stomata of the abnormal leaves does not compensate for the disadvantages of the genetic aberration caused by \( dr \). It is possible that the single gene \( dr \) causes the single abnormality in stomatal function and that all its other abnormalities arise therefrom. If this is true, hydration of the leaf must affect net photosynthesis per plant in other ways than by regulating stomatal opening and the entrance of carbon dioxide as the observations of Shimshi (2) have indicated.

### Literature Cited