Occurrence and transport and transport of a substance causing flowering in the Soya bean (Glycine Max L.)

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The question whether a substance causing flowering in plants exists, is still unanswered. In a recently issued publication Knodel [1]) once more puts the question quite clearly; there are two principal views on the matter: one, indicated by Knodel as the conception of Klebs, to the effect that flowering depends on a fixed ratio of soluble nitrogen to sugars in the plant, the other, first actually promulgated by Sachs, and assuming a specific substance causing flowering (Blütenbildender Stoff). Knodel shows in his investigation that there is no proof of the existence of a fixed ratio between N and sugars which causes flowering; that flowering may at all events occur with very different values of this ratio and that in the same species, with a particular value of the ratio, flowering is sometimes seen to occur and sometimes not.

The question what causes flowering is once more brought into prominence by the investigations of what we will here call the Russian school; this school, whose spokesman is especially Lysenko, but with whom various well-known physiologists, such as Ljubimenko, Rasumov, Maximov, Borodin, Dolgutschin, and others cooperate, has drawn the special attention of botanists to the possibility of influencing the life-cycle of plants; they base their opinion on prior investigations of Klebs, Gassner, Garner and Allard, etc. If it is possible to influence the lifecycle, as is shown by the processes which Lysenko summarizes under the name of "vernalization" to be actually the case, one is readily induced to think of specific substances, especially in the case of the so-called after-effect, which often brings about the transition from the vegetative phase to the reproductive phase weeks or months after the moment at which the factors which made this transition possible were present.

More especially the facts which are noticed in the photo-periodical reaction suggest the occurrence of such substances. The fact that a short-day plant reaches the reproductive phase when grown under a day of 12 hours, but does not develop flowers when grown under a normal-day (in our latitude), and that flowers are also produced when short-day is given only during a short period after germination, in our opinion clearly points in that direction. In his previously cited investigations, short-day and long-day plants were the very ones with which Knodel was unable to find any support for the view that it is a fixed ration between N and carbohydrates that causes

[1]) H. Knodel. Lässt sich die Klebs'sche Ansicht über die Abhängigkeit der Blütenbildung von der chemischen Zusammensetzung der Pflanze aufrechterhalten? Z. f. Botanik *29*, 449 (1936).

flowering. The very existence of an after-effect renders it in our opinion extremely unlikely that a particular ratio between assimilation products, formed long after the application of short-day, should be responsible for flowering.

And finally the discovery of growth substances leads us instinctively to the conception of specific substances causing flowering.

Various investigators have already tried by means of direct experiments or by deductions from observations made for other purposes to answer the question whether substances promoting or substances inhibiting flowering occur.

Direct experiments have been made by Krasnoseljskaja—Maximova [2]), who worked with winter-wheat. She tried to induce flowering (earing) by the application to the young plants of various sugars, asparagine and other N-containing compounds, yeast, etc., but without any result. No better result was obtained by injection into the young plant of an extract from flowering spring-wheat. When, however, an extract from germinating winter-wheat was injected into spring-wheat, a delay in earing was observed. From this observation Krasnoseljskaja draws the conclusion that in germinating winter-wheat a substance is present which inhibits flowering.

Sereiskii and Sluckaja [3]) find no confirmation of this conclusion in their own experiments. They cut germinating seeds of winter-wheat transversely and fixed half seeds of vernalized and unvernalized wheat tightly together with plaster of Paris. No interaction between the vernalized and the unvernalized halves was observed, although the united plants grew well; no substance is translocated from one half to the other. Not being able to read the original Russian papers, we used summaries in English, so that we can form no judgment as to the details of this work.

Garner and Allard [4]) have made some observations in connection with the problem; soya beans, *which had already developed flowers* after short-day treatment; were subsequently cultivated under long-day conditions. These plants grow on vegetatively and do not develop any further flowers. It is possible to assume from this that there is no specific substance, but there is also the possibility that the production has ceased and that the substance formed has been used up during flowering. There is, however, no cogent reason to assume the presence of a specific substance; it is also possible to explain matters by assuming differences in the entire metabolic process in short-day and long-day plants. The authors themselves do not discuss this point.

Some Russian investigators made experiments on the question whether the effect of vernalization is reversible or irreversible; whether a fitness to flower, once induced, disappears, and vegetative growth sets in, when, after the induction, the specific factors for

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[2]) T. A. Krasnoseljskaja—Maximova, Russian, An attempt to elucidate the internal causes of retardation of earing in winterforms. Trudy Prikl. Bot. *27*, 113 (1931).

[3]) A. Sereiskii and M. Sluckaja, Russian, On the nature of vernalization. Bot. Zurn. S.S.S.R.U. *19*, 311 (1934).

[4]) W. Garner and H. Allard, Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jrn. Agr. Res. *18*, 553 (1919/20).

induction are no longer applied. Ljubimenko [5]) cultivated soya beans under short-day conditions; when the plants flowered, they were transferred to long-day conditions. Shoots which developed under these long-day conditions did not flower; from this it is concluded that the main stem no longer has at this moment the capacity to produce reproductive organs, and that fitness to flower is reversible. If, however, a special substance be supposed to be the cause of flowering, it may be consumed by flowering in this case also, so that nothing is left to induce flowering in new shoots.

We may further mention experiments on localization of the photo-periodic effect. Rasumov [6]) speaks in his publication of "transport"; this implies in our opinion a substance to be transported. He works with Ullucus tuberosus and Solanum demissum, plants which show a reaction to short-day treatment in forming tubers, and, as far as Ullucus is concerned, by changes in the top of the sprouts. Rasumov darkens various percentages of the total leaf-surface, and states that only when a certain percentage is treated tuber-formation does start: the tuber-formation increases as a result of increasing the darkened portion above this limit. When only the middle part of a sprout is given short-day, the base and the top show a reaction; transport to the base is said to be better than that to the top. We think that no positive proof is given in these experiments of the presence of a transport substance.

Finally Garner and Allard [7]), working with Cosmos sulphureus, a short-day plant, darkened the top of the stem and treated the lower part with short-day. This part developed flowers, after which the top continued to grow, and the whole plant was exposed to normal day-length; the top then formed flowers also. The authors do not discuss this question, but we consider that the notion of a specific substance transported to the top of the plant suggests itself.

It will be clear from these facts that the problem whether specific substances are formed in vernalization or photo-periodic treatment is not yet solved; in many cases it was not even discussed; none the less we think that many indications pointing to its occurrence are present. A study of Hitchcock and Zimmerman [8]) gives in our opinion fresh support to this idea; they noticed that "synthetic substances" hastened flowering in a Turkish tobacco variety; this substance also inhibits growth, and it is worthy of note that in short-day plants short day causes not only flowering but also an inhibition of growth.

The first of the authors has worked for some years already on the vernalization of soya beans; as a result we have some varieties which have shown during 3 successive years that no flowers are produced under normal day-length when not sown before the end of April. They flowered in a 12—13 hour day. These species afford a good object for the study of the problem: does a substance

[5]) V. N. Ljubimenko, Russian, On the theory of artificial regulation of the length of the vegetative period of higher plants. Sov. Bot. No. *6* (1933).

[6]) V. J. Rasumov, Ueber die Lokalisierung der photoperiodischen Reizwirkung. Planta *23*, 384 (1935).

[7]) W. Garner and H. Allard, Localisation of the response in plants to relative length of day and night. Jrn. Agric. Res. *31*, 355 (1925).

[8]) A. E. Hitchcock and P. W. Zimmerman, Absorption and movement of synthetic growth substances from soil as indicated by responses of aerial parts. Contr. Boyce Thompson Inst. *7*, 447 (1935).

causing fitness to flower exist? It should be present in plants treated with short-day, and absent in those cultivated under normal-day. If such a substance should occur, its existence would be shown to be probable if one succeeded in transporting the fitness to flower by uniting a s.-d. soya (s.-d. = plant cultivated under short-day) with a l.-d. soya (l.-d. = plant cultivated under long-day) by grafting [9]). In this way we tried to answer the question.

Methods.

We used two varieties of soya bean: Ked. 29, obtained from the Algemeen Proefstation voor den Landbouw at Buitenzorg, and soya à graines jaunes, obtained in 1933 from Vilmorin. This latter variety will be indicated below as the yellow soya bean.

We first tried whether it were possible at all to graft the soya bean, and succeeded in making whip-graftings of young soya bean plants. The plants were cultivated in the open air, and proved to be in the best condition for grafting during a period of about 10 days before and after the appearance of the first flowers, at the age of about 50 days. Grafting was performed by making an oblique cut at one of the internodes above the insertion of the cotyledons, sometimes at the first internode, but preferably at the 3rd to the 5th. The young stems should be rather rigid; for this reason the plants should not be cultivated in a hothouse.

For our grafting experiments we used a support with clamp in which the stem-top, i.e. the scion, was gripped, in order to be able to press it against the cut of the stock. Two thin pieces of raffia were then tied round the two parts and the whole was covered with grafting wax. It proved to be of the greatest importance to keep transpiration at a low level during the first few days after the operation; the plants had to be kept for at least 10 days under a glass bell in a cool place and in weak light. We put them for that purpose in a greenhouse. Reduction of transpiration could also have been attained by cutting the leaves, but we dared not do so, as the leaves might possibly have something to do with the faculty of flowering.

After removal of the glass bell the plants stayed for another week in the greenhouse; it was then possibly to transfer them into the open air. We were finally able on several occasions to achieve complete success, but for this the utmost care is necessary, especially during the after-treatment. We got the impression that it is favourable when the under-stem retains some leaves, that the grafting is therefore not performed directly above the cotyledons, but higher up the stem; moreover the axilar buds of the leaves sprout better than those of the cotyledons, axilar branches being necessary to control the influence of the scion on the stock .

For the following experiments the yellow soya bean was used.

All the seeds were sown on May 5th; germination followed on May 10th; the young plants were transplanted on May 14th. The s.-d. group was placed on May 18th on a flat truck, which was drawn every day at 7.30 a.m. out of a darkened shed and pushed back again at 5 p.m. Thus the plants were given a short-day of 9½ hours; care was taken that the temperature did not rise too high in the

[9]) We are glad to mention that it was Prof. Dr. W. H. Arisz, of Groningen, who suggested the idea of grafting to us.

shed. The l.-d. plants were given the normal-day at 53° 14′ N.L.; on the day of sowing, May 5th, its length was already 15 hours.

On July 3rd 12 graftings were performed as follows:

6 s.-d. stocks were given a l.-d. scion,
6 l.-d. „ „ „ „ s.-d. „ .

After the operation all these plants were exposed to the length of the normal-day, although during the first-period to weak light only (see above).

Lenght of day.

Day-length in the latitude of Groningen (53°14′ N.L.) is as follows:

April	1	approximately	13.00	hours
„	15	„	14.02	„
May	1	„	15.04	„
„	15	„	15.59	„
June	1	„	16.42	„
„	15	„	17.06	„
July	1	„	17.06	„
„	15	„	16.40	„
Aug.	1	„	16.00	„
„	15	„	14.50	„
Sept.	1	„	13.50	„
„	15	„	12.50	„

We noticed that yellow soya beans, sown on March 29th, still formed flowers. It is known that especially on young plants the influence of a shortday is a strong one; it may therefore be assumed that these plants showed a reaction after the day-length they were exposed to between April 1st and 15th, a duration, that is, of from 13 to 14 hours. As in previous years, plants sown at the end of April or the beginning of May never showed a reaction.

There is thus a possibility that plants which are grown after September 1st in normal day-length (13.50 hours) might show a short-day reaction. Although in other experiments we had noticed that in full-grown plants the reaction on short-day (9½ hours) only becomes visible after about one month, we thought it better, in order to avoid false conclusions, to finish our experiments on September 15th.

Results

The plants grown continually in short-day all formed flowers; general flowering started on July 8th. Plants grown under normal-day did not show any signs of flowering, just as we had noticed the last three years. It may be regarded as certain that the long-day plants used for these grafting experiments would have behaved in the same way and would have formed no flowers at all.

At the moment, thus, when the grafting was performed, (July 3rd), the s.-d. plants already showed many flower-buds. The various plants of each series showed very much the same habit, a fact which was always noted in soya bean plants grown under the same conditions.

Our 6 plants with s.-d. stock and l.-d. scion (top) behaved in the following way:

Fig. 1. Grafted soya bean plants: left long-day stock, short-day scion; right short-day stock, long-day scion. × point of grafting; ➝ axil with flower photographed in fig. 2.

Fig. 2. Enlarged photo of axil with flowers on sidebranch of long-day stock, represented in fig. 1.

Fig. 3. Soya bean plants 70 days after germination; right grown under short-day (9½ hour), left grown under normal-day.

The leaves of the stock did not grow any more; often they were shed; new flowers did not develop; only in one plant a pod developed; side branches scarcely sprouted at all. The l.-d. scion, on the contrary, grew vigorously ,and looked just like a normal l.-d. plant. On September 15th one of these plants showed a flower-bud on the scion.

The behaviour on the 6 plants with l.-d. stock and s.-d. scion was totally different. The scion hardly grew at all after grafting, and no more flowers were formed on it; the leaves became yellow and often dropped off. In one plant the scion at length died and was thrown off on August 1st. The l.-d. stock, on the contrary, formed vigorous side branches. The poor growth of the scion may, of course, be due to the sprouting and growth of these side branches, but we could not cut these, since, if flowering developed, it would necessarily occur on these branches.

The side branches have the vegetative type, as may be seen from fig. 1.

About August 27th 3 plants had the first flowers on the side branches; 2 showed flower-buds, and the plant from which the scion was thrown off on August 1st showed a flower on September 15th. (Fig. 2). All these plants thus reached the reproductive phase. The top end of the branches shows a slight inhibition of growth, the internodia are a little shorter, the development of hairs is somewhat less. We think that these features may be considered as signs of the reproductive phase; they form, however, only a weak indication in that direction.

As stated above, not one of the control plants showed any sign of flowering till September 15th.

Conclusions.

It seems to us that no other conclusion can be drawn from these facts than that fitness to flower has a material carrier, which is transported from the s.-d. plants over the grafting point to the l.-d. plants. In one series the transport took place in the basal direction, in the other in the apical direction, but this happened only in one plant, so that there is no absolute certainty in this case. Where the direction was basal flowering started earlier and more generally (in all plants); basal transport seems to occur more easily.

Whether the material carrier should be considered as a specific flowering hormone, or another type of planthormone, or a specific carbo-hydrate or nitrogenous compound formed in the assimilation process, cannot be concluded from these experiments. It seems clear to us, however, that no fixed ratio N: carbohydrate can be considered as the cause of flowering, for the side branches and the scions have developed under exactly the same conditions as the l.-d. control plants; so that the same ratio did not have any such effect in these plants. It further follows from these facts that transport seems to take place better in the basal direction than in the other direction, which is in agreement with Rasumov's observation (see above). That transport actually does take place looks still more probable owing to the fact that flowering starts first in the first side-branch under the grafting point, at a point, that is, nearest to the place of origin, namely, the scion. We would also draw attention

to the fact that photoperiodicity in soya causes flowering in combination with inhibition of growth. Garner and Allard (1919/20) have already observed this, and in this variety also this phenomenon is pronounced, as may be seen in fig. 3. We therefore think that our observation of a slight inhibition of growth in the side branches is not without importance; it would seem to prove that both fitness to flower and inhibiton of growth are, in this case also, transported from scion to stock or vice versa.

There is a certain discrepancy between our observations and those of Lysenko (cited in Vernalization and Phasic Development of Plants, Imperial Bureau of Geneties, 1935). He made cuttings from flowering soya plants, which he cut above and below the place where flowers ware inserted. Those taken from above this place produced flowering plants, the other ones did not flower. In cotton plants, after flowering, side branches sprouted; those formed above the flowers yielded flowering sprouts, the lower ones did not. This seems not to agree with our observation of easier basal transport.

After the series of graftings about which we have reported above, other series were made in the latter part of July and during the month of August. In the middle of September no flowering was observed in these plants; for the reasons stated above we then had to finish this experiment, as results, however they might turn out, could not have been entirely reliable. This was regrettable, for in these series we also had control plants which differed from the grafted ones in one respect only, viz., a l.-d. scion was grafted on a l.-d. stock, in order to see whether the operation of grafting in itself would have any influence. It is, however, very unlikely that there would be any influence, since Garner and Allard and other investigators have already shown that no other environmental condition but the relative length of day and night has any effect at all.

Summary.

It is shown that in the soya bean (Glycine Max L) when a flowering scion is grafted on a stock treated with long-day, that is, without fitness to flower, flowers develop on side branches of the stock; conversely a short-day stock causes flowering in a long-day scion. A substance causing this phenomenon thus passes the point of grafting; transport in the basal direction seems to be much easier than in the apical direction, for flowers were formed on the long-day stock earlier and more profusely than in the converse case.

Groningen, September 1936.