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BREEDING OF NON-BITTER CUCUMBERS

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Abstract

A large number of seedlings of various cucumber varieties have been tested for bitterness. In the American variety Improved Long Green one plant was encountered that was non-bitter. This form of non-bitterness is highly valuable for breeding purposes, because of the possibility to select for non-bitterness at the seedling stage. Non-bitter varieties, as occur in other *Cucurbitaceae*, were not encountered in the many varieties investigated. Bitterness of cucumber and melon plants seems to be controlled by one dominant gene.

Plants can be judged for non-bitterness simply by tasting them, provided that the tasters have previously been tested for sufficient discriminatory power.

Non-bitterness of the young seedlings can also be shown by a chemical reaction. To this end a simple method has been developed.

Introduction

Bitter principles are of general occurrence in the *Cucurbitaceae* (Hegnauer, 9; Enslin, Joubert and Rehm, 4). They may occur in the roots, leaves and fruits. The seeds of most *Cucurbitaceae* are not bitter, but a few hours after germination very high concentrations of bitter principles may be encountered in the roottips (Rehm and Wessels, 12).

The occurrence of bitter fruits in cucumber, *Cucumis sativus*, can be a serious economic problem. According to the manner in which they form bitter principle cucumbers can be divided into three categories.

a. cucumber varieties of which the fruits can become bitter and of which the vegetative parts of the plant are always bitter. This category embraces most of the current varieties. The extent of bitterness of the fruits depends on the genetical characters of the varieties and on the growing conditions (Vogel, 13).

b. cucumber varieties of which the fruits, even under unfavourable growing conditions do not turn bitter, but of which the vegetative parts of the plants are always bitter. The German variety Reusrath's Bitterfreie belongs to this category. Some authors mention varieties which under unfavourable growing conditions never produce bitter fruits. However, they do not indicate whether the vegetative parts of the plant contain bitter principle. Probably these varieties also belong to category b. Hartmair (8) never found bitter fruits in some Asiatic cucumber varieties used for breeding work in Weihenstephan. Adam (1) mentioned non-bitterness in the fruits of various lines of Chinesische Schlangen; Enslin, Joubert and Rehm (4) never found bitterness of the fruits in newly developed cucumber varieties grown under S. African conditions.

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c. Cucumber varieties, which completely lack bitter principles both in the fruits and in the vegetative parts of the plant. Varieties with entirely non-bitter plants are not yet mentioned in the literature. REHM and WESSELS found non-bitter plants in other *Cucurbitaceae*, such as *Cucurbita maxima, Cucurbita mixta, Cucurbita pepo, Luffa cylindrica, Cucumis melo.* In the cucumber varieties investigated by them non-bitter plants could not be detected. They assume that if cucumber seedlings could be found that were free from bitter principle, then the plants raised from them would not form bitter principles under any conditions.

In recent years the problem of bitterness of cucumber fruits has become more and more serious in the Netherlands. That is why the Institute of Horticultural Plant Breeding at Wageningen started a series of investigations to find out whether this problem could be solved by breeding non-bitter varieties. At first only the variety Reusrath's Bitterfreie was available, of which the fruits only are non-bitter. Attempts were made to indicate this form of non-bitterness in the young plants by means of a chemical method (see page 16). After receiving in 1957 the above mentioned publication by REHM and WESSELS (12) the investigations of Reusrath's Bitterfreie were stopped. Instead a search was made for cucumber plants that dit not contain any bitter principles.

If non-bitter plants could be found it would be very advantageous for breeding work, for then it would be possible to select easily for non-bitterness in the young seedling stage. In populations from crossings with Reusrath's Bitterfreie, which variety has only non-bitter fruits, selection is not possible until the fruits are formed. Moreover the plants should be grown under such conditions that it becomes possible to discard those plants which can produce bitter fruits under unfavourable conditions. In this case selection is more difficult and less reliable.

To begin with, a large number of plants were tasted and at the same time attempts were made to develop a chemical method of distinguishing the non-bitter plants from the bitter ones.

CHEMICAL INVESTIGATIONS ON BITTER PRINCIPLES

a. Literature review

As a rule bitter principles are compounds of which the widely varying structure is not yet sufficiently known. Research on bitter principles in *Cucurbitaceae* was impeded because of the difficulty to obtain these compounds in pure crystalline form. Most investigators were unsuccessful in this respect, so that the data in the older literature are often contradictory. Recently more data have been obtained on these often very toxic bitter principles as a result of the work of some South-African investigators (ENSLIN, REHM *et al.* 3, 4, 5, 11 and 12).

An extensive literature survey has been written by HEGNAUER (9).

The isolation and chromatography of the bitter principles in *Cucurbitaceae* was investigated by ENSLIN (4). ENSLIN calls these bitter principles cucurbitacins; he distinguishes cucurbitacin A, B, C etc.

In about 90 of the approximately 800 known species of the *Cucurbitaceae* bitterness has been reported. *Cucumis sativus* contains cucurbitacin C. The young seedlings contain cucurbitacin B in the roots, whereas the cotyledons at first contain B and C, later only C. The young seedlings of *Cucumis melo* contain cucurbitacin B in the radicles and cotyledons; in addition traces of other bitter principles occur. According to ENSLIN and RIVETT (5) many cucurbitacins occur as glycosides. All the members of the genus *Cucumis*; however, contain the cucurbitacins as aglycones (11). The tentative formula for B is $C_{32}H_{48}O_8$, that for C is $C_{32}H_{50}O_8$. All bitter principles in this family contain two or more alcoholic hydroxyl groups (in cucurbitacins A, B, C and E an acetyl group is also present) while a very characteristic structural feature is the presence of several keto-groups.

b. Experimental results

When in 1956 our chemical investigations of bitter cucumbers were started, a large part of the literature mentioned above had not yet been published. At first our aim was to find a simple chemical method of distinguishing in a young stage cucumber varieties which can produce bitter or non-bitter fruits. We started with a search for a reaction to bitter principle in the fruits. A large number of reactions with press-juice from bitter and non-bitter fruits did not produce any reproducible difference. After having taken note of the publication of ENSLIN *et al.* (4) we started a paper chromatographic investigation of a number of young plants. To this end a variety was used of which the full-grown fruits were known to become easily bitter as well as a variety (Reusrath's Bitterfreie) with non-bitter fruits. Of a number of plants of both varieties a purified extract was made (3), and after paper chromatography the reaction of ENSLIN with copper acetate-potassium permanganate was applied. In addition an infra-red-absorption spectrum of these purified extracts was made by Mr. L. VAN DIJCK, of the N.V. Organon at Oss. No reliable differences were found.

Treatment of the chromatograms with various reagents did not produce any differences either. Some reactions were positive, to be sure, but they were so in extracts of both varieties. For instance treatment with $SbCl_3$ in chloroform after heating to 100 °C produced clearly fluorescent spots under U.V. light.

In both cases the copper acetate-permanganate reaction according to ENSLIN was also positive. Moreover the Rf values of all spots were the same so that the provisional conclusion could be drawn that the $SbCl_3$ reaction is a reaction to cucurbitacin C. This reaction is more sensitive than ENSLIN's.

Cucurbitacin C was demonstrated in the vegetative parts of all young plants under investigation. Differences in composition between plants with non-bitter and those with bitter fruits were not found.

At the end of 1957 a search was started for varieties with non-bitter seedlings. At that time it was of importance to develop a rapid reaction to cucurbitacin C. Such a reaction should also be simple and highly sensitive, since the bitter principle content in the cotyledons is only 0.001–0.009 % (12). A reaction necessitating previous purification is not usable. Hence, a reagent like triphenyl tetrazoliumchloride is not suitable, since other plant constituents also react with this reagent. Finally it was found that the bitter principle can be demonstrated without chromatographic separation by extracting a cotyledon with chloroform, placing a drop of the solution on filter paper, moistening the paper with a saturated solution of $SbCl_3$ in chloroform and heating at 100 °C for 3 minutes. The result will be a lilac fluorescent spot, which is visible in

U.V.light. The same reaction with radicles of bitter seedlings produced a weak cinnamon spot in U.V.light. Using the pure cucurbitacins B and C, which we had meanwhile received from Dr. REHM, these reactions could be confirmed. According to ENSLIN *et al.* (4) the bitter principles produce only weak spots with this reagent. From correspondence with the South African investigators it has meanwhile appeared that Dr. ENSLIN has been able to confirm that cucurbitacin C reacts much more markedly with $SbCl_3$ than the other cucurbitacins.

Young seedlings of a variety of *Cucumis sativus* from Celebes with a variable bitter principle content were used for comparing the above method with the tasting tests. Fifty seedlings were investigated of which the majority were positive; some were slightly positive and the remainder negative. The results of the tasting tests were identical. Later it was found that in the older plants, which were negative or slightly positive in the seedling stage, bitter principle did occur. So neither the taste nor the chemical reaction of the young seedlings fully guarantee the absence of bitter principle in older plants.

For selection purposes the reaction is carried out as follows.

> In a metal rack 25 test-tubes are placed. This rack has been made in such a way that the tubes cannot shift about and consequently need not be numbered. In each tube one-half of the cotyledon is placed, which operation does not harm the plants. Next about 0.7 ml of chloroform is poured from a burette into each tube, after which the rack is placed in a water bath at about 90°C for some minutes. The bitter principle is extracted by the chloroform, at the same time concentrating the solution to about one drop. Then the rack is removed from the water bath and from each tube a drop is run off on a piece of filter paper previously provided with 25 numbers. This paper is moistened with a saturated solution of $SbCl_3$ in chloroform, heated in a stove at 100°C for 3 minutes and judged under a U.V. lamp.

The intensity of the fluorescence is a criterion of the bitter principle content. Using a standard solution we found that cucurbitacin C can be demonstrated from about 0.01 γ (1 γ = 0.001 mg). So the reaction is very sensitive. We have tried in different ways to simplify the reaction, for instance by pressing the cotyledons on filter paper, drying, and reacting directly with $SbCl_3$ on the spots that have appeared. In this case, however, the results are indistinct and unreliable. In the radicles of seedlings with non-bitter cotyledons from a selfed non-bitter plant of Improved Long Green, bitter principle could not be demonstrated either. A chromatographic investigation of some 3 fruits of a non-bitter plant of the above variety showed that these fruits did not contain bitter principle or allied compounds. "Normally" non-bitter fruits often produce on the chromatogram a positive $SbCl_3$ reaction consisting of a spot with about the same Rf value as that of cucurbitacin C. Possibly this is a precursor of the bitter principle or a compound formed by enzymatic transformation from this precursor. This substance could not be demonstrated in *all* cases. Whether this is due to these compounds being absent or to their concentrations being too low is not clear. At any rate the $SbCl_3$ reaction is not suitable for determining bitterness in fruits.

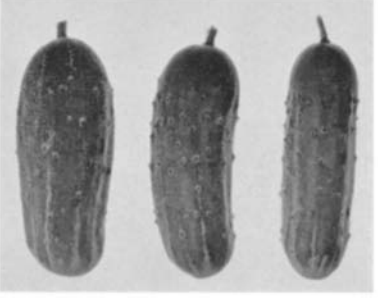

FIG. 1. FRUITS OF NON-BITTER IMPROVED LONG GREEN

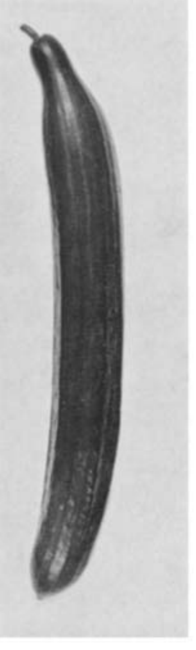

FIG. 2. DUTCH CUCUMBER VARIETY

SEARCH FOR NON-BITTER CUCUMBER PLANTS

It was ascertained whether the collection of cucumber varieties of our Institute contained any non-bitter plants. At first no rapid chemical method was available for distinguishing the non-bitter plants from the bitter ones. Therefore tasting them was the only usable method. Although a large number of plants can be rapidly tasted, there are some drawbacks. To test the discriminatory power of the tasters, use was made of a number of melon seedlings most of which were bitter (var. Honey Dew). Per twenty seedlings two non-bitter ones were included (var. Hales Best). A relatively small number of people working at our Institute was found to be capable of picking out correctly the plants that were non-bitter. As a rule a good taster cannot treat more than 20 to 30 plants at a time. The taster has to correct his taste now and then by consuming for instance a piece of toast or apple. The plants were judged for bitter principle content when the two cotyledons were in a horizontal position. (According to REHM and WESSELS (12) the bitter principle content of the cotyledons increases steadily until full expansion has occurred.) As the non-bitter plants, if any, had to be grown on, the tasters were not allowed to use more than one-half of a cotyledon. First it was ascertained whether, just as in *Cucumis melo* and other *Cucurbitaceae*, cucumber varieties with entirely non-bitter plants were already in our possession. Of the following varieties a number of plants, mostly some dozens, were tasted; among these, non-bitter plants were not found.

Acur, Baarlose Nietplekker VI en VII, Beemster Ruige, Beit Alpha Ben Shemen, Bertschagovskie, Betuwse Witte, Borovskie, Burpee's Sunnybrook, Butcher's, Cavallius Lage agurk, Chinese Lange Groene, Colorado 1414, Dansk Asie, Westlandse Halflange Witte, Delicatess, Delizia, Detroit, Driv Vestervang, Early Fortune 11123, Eminent, Evergreen, Excelsior, Favorit, Filia O.J.O., Fournier, Géant de pleine terre, Gele Tros, Graf Zeppelin, Guntruud, Highmoor, Hoffmann's Giganta, Hoffmann's Produkta, Ideaal, Izumi, Jeep, Kanmore, Kooperator, Lange Groene vollegrond, Langelands Riesen, Lange Witte, Marjalanskie, Medio Largo, Melnik, M.R. 17, Muromsk Drue, Niagara, Ohio 31, Orion, Pepino verde largo Inglese, Perfecta, Reusraths Bitterfreie, Rhinsk Drue, Robusta, Russisk Drue, Spangsberg grøn, Succes, Talachovskie, Tatschenskie, Toogood's Prolific Ridge, Torpedo, Unicum, Venus, Verbeterde vollegrond, Verde Paulistana 1 A.C.1306, Vert demi long Prolifique, Vert demi long Robusta, Vert long anglais épineux, Vert long Danois, Vert long Maraîcher, Vert long Prolifique, Vert très long de Chine, Volltreffer Kastengurke, Westlandse Gele, Wisconsin S.M.R. 12, Wisconsin S.R. 6, Wisconsin S.R. 10, Witte Bruid, Wjasnikowsche, Yamatosanjyaku, Yates Crystal, York State Pickling, Znojmo., I, II, III, IV, V (Roumania), F1 21954 (Drivgurka), a number of F1's of which both parents are shown in the above list.

Therefore it seemed more probable that, even when entirely non-bitter plants should occur, their number among the existing varieties would be very low. As we did not know what varieties were likely to contain non-bitter plants, a start was made with tasting a large number of seedlings of cucumber varieties of which we happened to possess much seed. Some of these seedlings were less markedly bitter, others were non-bitter or hardly so (see table 1). When grown on in a glasshouse the vegetative parts of all plants but one were not entirely free from bitter principle. Although under our conditions none of these plants produced bitter fruits, all the plants that had traces of bitter principles in their vegetative parts were removed. At the end of the growing season only one out of the 15,000 plants tested remained. Neither in the vegetative parts nor in the fruits of this plants could bitter principles be demonstrated by organoleptic or chemical methods. This plant, which was found in the American variety

Improved Long Green, is being used for further breeding work as size and shape of the fruits are unsatisfactory for our conditions (fig. 1 and 2).

TABLE 1. RESULTS OF TASTING A NUMBER OF SEEDLINGS OF CUCUMBER VARIETIES

Varieties	Number of plants tasted	Non-bitter or slightly bitter
Amsterdamse Gele	124	–
Ballady short fruited	250	–
Burpee Hybrid	154	–
Cuc. sativus, Cairo	17	4
Cuc. sativus, Celebes	1130	115
Cuc. sativus, Egypt	460	9
Cuc. sativus, India no. 1	160	–
Cuc. sativus, India no. 2	87	–
Cuc. sativus, India no. 3	67	–
Cuc. sativus, India no. 4	470	1
Cuc. sativus, Indonesia no. 1	550	–
Cuc. sativus, Indonesia no. 2	440	22
Cuc. sativus L., 2064, Leningrad	660	–
Cuc. sativus L., 2221, Leningrad	460	–
Dlinnoplodnyj	640	–
Groene Standaard	176	–
Improved Long Green	1620	2
Kagaoanaga-fushinari	10	1
Klinsky	240	–
Leningradskij 23	425	–
Lentse Gele	188	–
Mirovskij	133	–
Njeschin	520	–
Palmetto	200	–
Poona, Short White	225	–
Rjäbtschik	450	–
Rschaw 301	580	–
Spiers	341	–
Spotvrije	243	–
Telegraaf	208	–
Toogood's Her Majesty	19	1
Wladiwostok	580	–

HEREDITY OF BITTER PRINCIPLE

F_1 plants from a cross of non-bitter Improved Long Green and a bitter Dutch variety were all bitter. The seeds obtained after selfing of the non-bitter Improved Long Green plant produced only non-bitter seedlings.

In the F_2 plants derived from a cross between non-bitter Improved Long Green and a bitter Dutch variety, the ratio between the bitter and non-bitter plants was found to be 983 : 344. This does not differ significantly from the ratio 995 : 332 representing the correct 3 : 1 segregation.

The F_2 plants were tested chemically. The non-bitter plants were then subjected to a tasting test in which they did not show bitterness either.

As we happened to have F_2 seeds of a cross between the melon varieties Westlandse Enkele Net with bitter seedlings and Hales Best 36 with non-bitter seedlings, we were also able to test the ratio bitter: non-bitter in melon. 297 plants were tasted, 225 were bitter and 72 non-bitter. Here, too, the figures point to a bitter/non-bitter ratio of 3 : 1.

DISCUSSION

The assumption of REHM and WESSELS (12) that entirely non-bitter cucumber plants exist was found to be true. However, non-bitter varieties, as occur in melon and other *Cucurbitaceae*, were not encountered in the many varieties investigated.

As already stated in the introduction non-bitterness of the whole plant is very advantageous for breeding purposes, for then it is possible to select for non-bitterness in the young seedling stage.

Bitterness of the plants seems to be controlled by one dominant gene. BARHAM (2) found that the extreme bitter taste of the fruits of the wild Hanzil Medicinal cucumber of India depends on one dominant gene. PATHAK and SINGH (10) found the same mode of inheritance of the bitter principle in the fruits of *Lagenaria leucantha;* GREBENŠČIKOV (6, 7) in the fruits of *Cucurbita pepo;* ARASIMOVIC (cited in 6) in the fruits of *Citrullus vulgaris*.

It would be interesting to determine the mode of inheritance of varieties with bitter plants and non-bitter fruits (e.g. Reusrath's Bitterfreie) by crossing them with entirely non-bitter plants. REHM and WESSELS (12) presume that in varieties with only non-bitter fruits bitterness in the fruits is controlled by another genetic mechanism which suppresses the occurrence of cucurbitacins in the fruits of the non-bitter forms in the presence of the gene responsable for the formation of bitter principle. Only those varieties which have non-bitter seedlings lack the gene for bitterness.

We cannot yet explain the fairly large differences in the bitter principle content of the seedlings of some origins of *C. sativus* investigated by us.

In our trials neither the taste nor the chemical reaction of the young seedlings always guaranteed the absence of bitter principle in older plants. Very likely some seedlings were erroneously considered as non-bitter, because their bitter principle content was not high enough to fall within the sensitivity range of both the chemical and organoleptic method.

Therefore checking the bitter principle content of the selected seedling at a later developmental stage is necessary. If in breeding the number of plants used is not too large, then tasting the cotyledons of the seedlings is the simplest and most rapid method, provided one or more capable tasters are at hand. If thousands of plants have to be tested the chemical method will generally be preferable.

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SAMENVATTING

Het kweken op bitterstofvrijheid van komkommers

Een groot aantal zaailingen van verschillende komkommerrassen werd getoetst op bittervrijheid. In het Amerikaanse ras Improved Long Green werd één plant aangetroffen, die geen bitterstof bevatte. Deze vorm van bitterstofvrijheid is zeer waardevol voor veredelingswerk omdat het hierdoor mogelijk is reeds op bitterstofvrijheid te selecteren in het zaailingstadium. Bitterstofvrije rassen, zoals die bij andere *Cucurbitaceae* wel voorkomen, werden niet in het onderzochte sortiment aangetroffen.

Bitterheid van komkommer- en meloenplanten lijkt bepaald te worden door één dominante factor. De toetsing op bitterstofvrijheid kan organoleptisch eenvoudig worden uitgevoerd, mits vooraf de proevers op hun smaakvermogen zijn getoetst.

De bitterstofvrijheid van jonge plantjes kan ook chemisch worden aangetoond. Hiertoe werd een eenvoudige methode uitgewerkt.

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