actions increasing further at higher temperatures. Compared to reactions at isotherm conditions, these increases amount to about 25% when the thermode is 3 °C warmer, and 35% at 5 °C more. Here too, subsequent experiments with the same mechanical pre-tension and isotherm thermode show the values initially received. Observations by BARDACH[5], who did not get any reactions to isotherm touches after temperature conditioning, confirm this. As the mechanical force of the stimulus was not changed here, and the reaction increased right after the beginning of the new stimuli combination, this change of reaction must be associated to the changed temperature.

The reactions of a single animal to the various stimuli combinations are represented in Figure 2. Each column consists of about 50 measurements.

On the basis of these results, one has to inquire about the significance of the temperature component in these stimuli combinations. The stronger reactions with heated thermode are underlined by the fact that, according to the *t*-test by Student[9], the differences between the 4 pairs of stimuli combinations are significant (0.27%-level), except for the last one (see Figure 2).

According to electrophysiological investigations of the fish skin[7], mechanical stimulation and simultaneous heating lower the reaction within the skin nerve. This corresponds to a cold-receptor-characteristic of those mechanoreceptors. It can be assumed that the same characteristics are present in behaviour experiments. Therefore the increased reaction of the goldfish can be seen as compensation of the reduced skin nerve activity. However, this effect has to be coupled with a simultaneous temperature perception, since, 1. there is no evidence so far that there is a special receptor system for temperature in fish, and 2. they can still be well conditioned to small temperature differences as low as 0.05 °C[10,11]. It is interesting that stronger pressing of the fish at heated thermode corresponds with observations of WEBER[12] who noted that warm subjects seem lighter for man.

In which way temperature information is effectively perceived and processed in the CNS has to be clarified by further investigations.

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The Effect of Sugar Nutrition of in vitro Pollinated Placentae on Seed Set and Dormancy in *Nicotiana tabacum* L.

V. BALATKOVÁ, E. HRABĚTOVÁ and J. TUPÝ

Department of Genetics, Institute of Experimental Botany, Czechoslovak Academy of Sciences, Flemingovo nám. 2, CS-160 00 Praha 6 (Czechoslovakia), 5 April 1976.

Summary. The substitution of sucrose in the nutrient medium for in vitro pollinated placentae by glucose and fructose favours seed formation and considerably enhances the germination of seeds just attaining maturity. This effect of invert sugar was wholly suppressed in the mixture with two parts of sucrose.

The pollination of excised placentae cultivated on artificial media results in ovule fertilization and viable seed formation[1,2]. In certain cases, the seeds formed in vitro germinate directly on placentae[2–6]. However, very often no exact data concerning the period between seed maturation and the beginning of germination are given. In our experiments with *Nicotiana tabacum*, seed germination in situ varies from experiment to experiment and in some cases all seeds dry out and pass to a dormant state as under natural conditions. For test-tube fertilization, sucrose has been the only sugar component of the medium so far used. Its substitution by glucose and fructose is shown here to affect seed formation and seed germination on placentae.

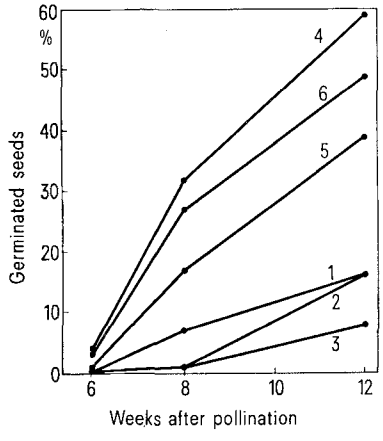

Fig. 1. The course of germination of seeds formed on excised placentae cultivated on various media: 1 and 2, media with sucrose (0.15 *M*); 3, medium with sucrose (0.1 *M*) + glucose (0.025 *M*) + fructose (0.025 *M*); 4, medium with glucose (0.075 *M*) + fructose (0.075 *M*); 5, medium with glucose (0.1 *M*) + fructose (0.05 *M*); 6, medium with glucose (0.05 *M*) + fructose (0.1 *M*). All media were sterilized under normal pressure, except the medium 2 which was autoclaved.

Material and methods. Experimental plants of *Nicotiana tabacum* L., cv. Samsun were grown in free soil in a glasshouse. The techniques of excision, pollination and cultivation of placentae were the same as described earlier[7]. The basal medium prepared according to NITSCH[8] was supplemented by White's vitamins and 0.05% casein hydrolysate, the final concentration of sucrose being 0.15 *M*[2]. The medium was either autoclaved for 20 min at 0.15 MPa or sterilized under normal pressure successively

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twice at 100 °C for 30 min. The latter mode of sterilization was also applied to the media where sucrose was replaced by sugar mixtures.

The placentae were cultivated at 25 °C in the dark for 12 weeks after pollination. The experiments were performed in 2 sets, each variant involving 20 ovaries. The results were evaluated statistically using the analysis of variance and Duncan's test.

Results and discussion. Germinating seeds in situ appeared as early as 6 weeks after pollination, but only in those media, which contained a mixture of glucose and fructose without sucrose (Figure 1). During further 2 weeks of cultivation, the number of germinated seeds considerably increased (up to 32% in the case of medium with invert sugar) and some germinating seeds also occured in the media with sucrose. The highest germination during the whole period from the 6th to the 12th week after pollination was registered on the medium containing a mixture of equal parts of glucose and fructose (medium 4). Shifting this proportion either in favour of glucose (medium 5) or fructose (medium 6) caused a decreased in the rate of germination. Statistical analysis of the percentage of germinated seeds 12 weeks after pollination revealed significant differences between the 3 media with sucrose and media 4, 5 and 6 and showed the highest germination on medium 4 (Figure 2B). There is no significant difference between media 5 and 6 and between the autoclaved and non-autoclaved sucrose media. It is of interest that invert sugar did not affect the germination when applied in a mixture with 2 parts of sucrose. On the other hand, glucose or fructose when applied alone, had a detrimental effect on the formation of seeds: in most ovaries no seeds developed, quite exceptionally a few seeds were set (unpublished data).

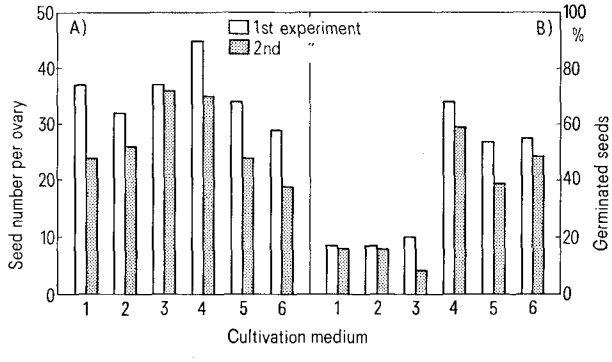

Fig. 2. Mean number of seeds per ovary (A) and percentage of in situ germinated seeds (B) 12 weeks after pollination of excised placentae in vitro. For media description see Figure 1.

Regarding the mean number of seeds per ovary, a similar situation was found as in the case of seed germination in situ (Figure 2A). However, the statistical treatment of the data showed that only medium 4 containing invert sugar significantly stimulated seed set with respect to the control media containing only sucrose.

Although the 2 experiments were performed within 1 week, the number of seeds per ovary, as well as seed germination was higher in the first experiment than in the second one (Figure 2). We assume that this may be related to some differences in the physiological state of the ovaries caused by a variation of environmental conditions such as luminosity and temperature.

It is generally accepted that sucrose is the best sugar component for media used in plant cell, tissue and organ culture. This is based on many experiments made with various plant parts and species in which the growth effect of different sugars was compared. The excized embryos of most plants[9] and young seedlings of tobacco[10] were found to grow in vitro better in sucrose-containing media than in the presence of other sugars, including glucose and fructose. Our results coincide with these observations in that sucrose cannot be replaced either by glucose or by fructose. They show, however, that each of these monosaccharides has a specific importance for the nutrition of placentae and for the development of ovules into mature seeds, and when supplied in a mixture as invert sugar, they bring about even better seed formation than the medium with sucrose. On the other hand, in pollen tube culture[11] and in barley embryos[12], sucrose could not be fully replaced by the mixture of glucose and fructose.

Regarding the stimulatory effect of invert sugar on seed germination on placentae, no similar observations have to our knowledge been made so far, and for its understanding further investigations are needed.

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'Binary Fission' and Budding in Microspores of *Heliconia bihai* L.

P. M. MATHEW and N. OMANAKUMARI[1]

Department of Botany, University of Kerala, Karyavattom, Trivandrum (India), 12 September 1975.

Summary. In *Heliconia bihai* L. 'binary fission' and budding occur in vivo in microspores which arise from diads, by furrowing of their wall. Frequency of this abnormal phenomenon considerably increased in materials subjected to cold treatment.

The course of development of the male gametophyte is remarkably uniform in angiosperms[2], barring a few unusual situations as in *Hyacinthus*[3], *Ornithogalum*[4], *Petunia*[5] and *Ophiorrhiza*[6]. This note deals with the occurrence in vivo of certain developmental abnormalities in microspores of *Heliconia bihai* L. (Musaceae).

Meiosis and stages of development of microspores were studied from flower buds collected from plants growing in field conditions as well as from inflorescences exposed to cold treatment (10–15 °C) for 24–36 h. Meiosis in the species is found to be normal with the formation of 12 bivalents at M I, except for failure of second division in 10–15% of the PMC's, and as a result, both tetrads and diads are formed at the end of meiosis. The microspores that have developed from the diads are larger in size (45 µm in diameter) than those developed from the normal tetrads (32 µm). About 30 h after meiosis, the nucleus of the larger microspores divides to form 2 equally sized daughter nuclei, while that of the normal microspores remain undivided (Figure 1). Following this, the larger bi-