

Development of Complete Homozygotes of Tobacco

Abstract. True homozygotes of *Nicotiana tabacum* L., herein referred to as Ky Iso lines, are being developed from haploids. A technique is described whereby seedlings resulting from fertilization are eliminated, leaving only suspect haploids. After confirmation, haploids are treated to double the chromosomes and these result in completely homozygous plants. One complete homozygote, Ky Iso 1 Ky 16, has been developed and shows extreme uniformity in all characters studied. The Ky Iso lines will be useful in genetic studies that require no plant-to-plant genotypic variability. The Ky Iso lines will be made available upon request as they are developed.

Cultivated tobacco (*Nicotiana tabacum* L., 24 chromosomes) is an amphidiploid of *N. sylvestris* (12 chromosomes) and *N. tomentosiformis* (12 chromosomes) or of *N. otophora* (12 chromosomes), both members of the section *Tomentosae* of the genus *Nicotiana*. Studies by Goodspeed (1) have shown that crosses of tobacco to either precursor species yields a high frequency of 12II (12 bivalents) and 12I (12 univalents) at the first metaphase of meiosis. Cytological evidence indicates that pairing between chromosomes of the precursor subgenomes is extremely low (2) but if it does occur it is probably due to segmental homologies. Metaphase I plates of 24I have been observed in the haploids herein discussed (Fig. 1). Thus, cytogenetically, *N. tabacum* can be thought of as a diploid.

Haploid *N. tabacum* plants have recently been observed (2, 3). The

production of *N. tabacum* plants that are completely homozygous by doubling the chromosomes of a haploid has been the object of the studies at this laboratory. The usefulness of the doubled haploids in genetic, plant pathological, physiological, and biochemical studies or as controls for defining various techniques is obvious. Stocks of doubled haploids are being increased so that they may be made available to interested investigators.

The technique for selecting haploids restricts their production to those lines or varieties that are susceptible to the tobacco mosaic virus (TMV). The TMV-susceptible line is used as the female parent in crosses to any line that carries the dominant, *N. glutinosa*, hypersensitive factor for TMV. The resulting seedlings are inoculated by the technique described by Stokes (4). Since the hypersensitive reaction is dominant, all seedlings that result from fertilization are killed by the hypersensitive reaction after the TMV inoculation (Fig. 2). The survivors (TMV susceptibles) are transplanted and reared to maturity. They are then checked for pollen fertility and those that are fertile are discarded since they are diploid and their origin is in doubt. Those that are sterile are saved as suspect haploids and examined for the presence of 24I at the first metaphase of meiosis (Fig. 1).

Eight haploids have been found and are being treated with colchicine to double the chromosome number. Over 100 haploid suspects are yet to mature. Two haploids have been doubled by the technique described. One doubled haploid developed from the burley variety Ky 16 and designated as Ky Iso 1 Ky 16 has been increased and is now available (5). The other,

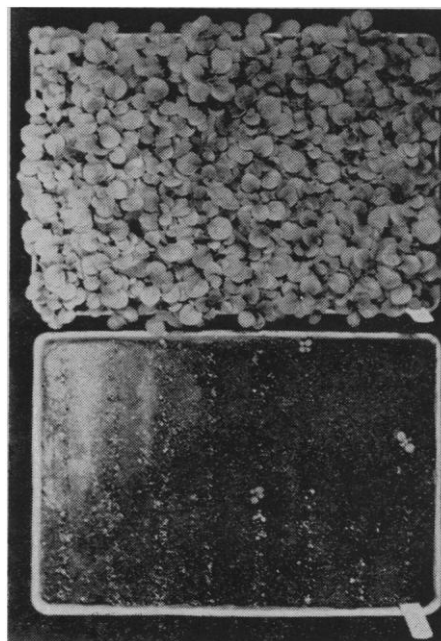


Fig. 2. Selection of suspect haploids by using hypersensitivity to tobacco mosaic virus as the seedling marker. Both pans were seeded at the same time. (Top) Control. (Bottom) Three suspect haploids remaining after selection.

Ky Iso 3 Burley 37, was recently developed but will not become available until 1964.

Ky Iso 1 Ky 16 was grown in the field in 1961 and 1962. The seedlings germinated and grew uniformly. Seed of ten plants was collected and a progeny of each was analyzed for total alkaloids. Total alkaloid studies on 6-week-old plants indicated that there was no significant difference in alkaloid level between these lines (6). Ky Iso 1 Ky 16 is a nonconverter line with low quantities of nornicotine (7). Plant response to controlled environment has been extremely uniform when used as a control in disease reaction studies.

In any study that demands a constant genotype, or in which plant-to-plant variation must be limited to micro-environmental response, the Ky Iso 1 Ky 16 line would be extremely useful.

The production of the available Ky Iso lines has been slow and tedious. Suspect haploids of a number of tobacco varieties that include burley, flue-cured, and dark tobacco types are now on hand. As they are identified as haploids, attempts will be made to double their chromosome number. The Ky Iso lines will be available upon request to investigators (8).

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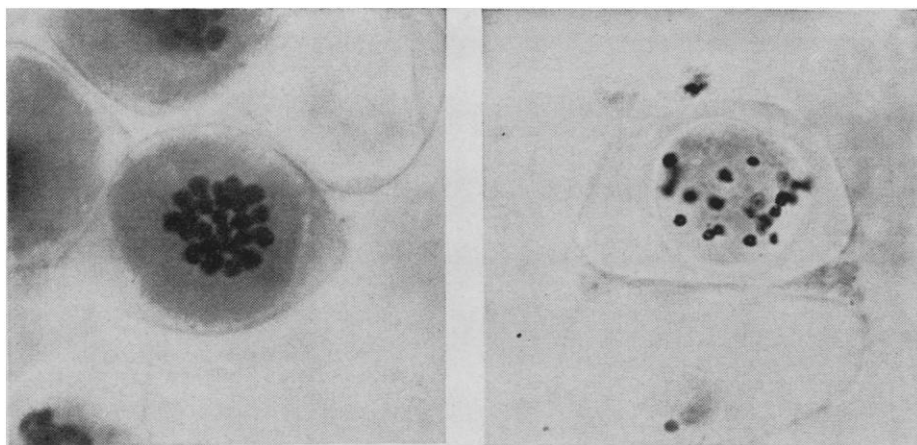


Fig. 1. Pollen mother cells of tobacco. (Left) Normal tobacco with 24II. (Right) Haploid tobacco with 24I. Note absence of metaphase plate in the haploid.

References and Notes

1. T. H. Goodspeed, in *The Genus Nicotiana* (Chronica Botanica, Waltham, Mass., 1954), p. 239, table 26.
2. D. deNettancourt and G. W. Stokes, *J. Heredity* **51**, 102 (1960).
3. L. G. Burk, *ibid.* **53**, 222, (1962).
4. G. W. Stokes, *Tobacco Sci.* **3** (1959), 129 (1960).
5. True homozygotes of *Nicotiana tobacum* L. are herein referred to as Ky Iso lines.
6. Data supplied by the research department of the Brown and Williamson Tobacco Corp., Louisville, Ky.
7. Data supplied by Dr. Eugene Glock, department of research and development, American Tobacco Co., Richmond, Va.
8. This study was in connection with a project of the Kentucky Agricultural Experiment Station, and is published with approval of the director.

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Stress Modification of Drug Response

Abstract. *Rats stressed by unilateral hind-leg ligation show a significantly decreased pharmacological response to the effects of hexobarbital, meprobamate, and pentobarbital. This effect is dependent upon an intact pituitary-adrenal axis, since it is absent in both hypophysectomized and adrenalectomized animals. It can be simulated by intravenous administration of adrenocorticotrophic hormone or corticosterone. The effect of stress on drug response is not noted with barbital, a compound which is not metabolized; this effect is inhibited by treatment of the animal with a drug metabolism inhibitor such as SKF 525-A (diethylaminoethyl 2,2-diphenylpentanoate). Compounds which can stimulate adrenocorticotrophic hormone secretion or act directly on the adrenals to produce corticosteroids should thus be able to stimulate their own metabolism or that of other drugs.*

Physiological stress, whether initiated by physical insult such as immobilization, ligation, or radical surgery or by chemical stressors such as formalin, reserpine, chlorpromazine, or histamine (1) leads to activation of the pituitary-adrenal axis as evidenced by an increase of adrenocorticotrophic hormone (ACTH) in the blood, a decrease of ascorbic acid in the adrenals, and, in the rat, an increase of corticosterone in the blood (2). While a host of clinically useful drugs act as chemical stressors, the effect of the stress response on drug action has not been investigated. Although adrenalectomized animals show a marked increase in duration of action of certain drugs, presumably because of decreased drug

metabolism (3), little is known of the significance of the pituitary-adrenal system as a physiological mechanism regulating the duration of drug response. Intensification of the normal functioning of this system by a stress situation has been chosen as the means of investigating its interaction with drug responses.

Male Holtzman rats were stressed by unilateral hind-leg ligation (rubber band around triceps muscle at distal end of the femur) for 2.5 hours at which time depletion of ascorbic acid in the adrenals was at a maximum. At such a time, after chemical stressors, blood corticosterone in the rat has been reported (2) to rise to a peak. Groups of control and stressed animals were administered hexobarbital (100 mg/kg), meprobamate (300 mg/kg), pentobarbital (35 mg/kg), or barbital (250 mg/kg) by intraperitoneal injection. Barbiturate and meprobamate "sleep times" were measured as the criterion of drug response. Sleep time was taken as the period of loss of the righting reflex. With the exception of barbital, a highly significant decrease in the duration of action of all drugs studied was seen (Table 1).

With the sleep time associated with hexobarbital as the criterion of drug response, adrenalectomized and hypophysectomized rats were subjected to the stress situation. No effect was noted ($p > .5$) on the duration of drug action.

On the other hand, treatment of intact rats by intravenous injection of ACTH (100 mU per animal) or corticosterone (50 μ g per animal) before hexobarbital treatment simulated the stress condition in that the duration of action of hexobarbital was significantly ($p < .0005$) decreased. Controls received injections of solvent, in equal volume, intravenously prior to injection of hexobarbital. Similarly, treatment of animals with 50 mg/kg of SKF 525-A (diethylaminoethyl 2,2-diphenylpentanoate) completely blocked ($p > .4$) the effect of the stress con-

dition on the duration of action of hexobarbital. That this compound inhibits drug metabolism is well known (4).

These data suggest a physiological mechanism for regulating the duration of drug response in the intact animal. Results obtained with barbital and with SKF 525-A suggest that the stress effect on the duration of drug action is mediated through increased drug metabolism.

In order to examine this premise further, rats were rendered functionally hypophysectomized by a 4-day treatment with morphine (5), and their ability to respond to the stress situation as well as to exogenous ACTH was determined. This sequence is of especial interest since prolonged morphine treatment is also known to depress drug metabolism (6). Hexobarbital sleep time was again used as a criterion of drug response. There are no significant differences in the duration of action of hexobarbital between the "morphine control" group and the "morphine stress" group, whereas the morphine-treated animal shows the characteristic response to exogenous ACTH. This would be expected since adrenal responsiveness is not altered (5) in the morphine treated animal, but the pituitary is functionally ablated. The action of morphine in depressing drug metabolism in these experiments is suggested by the highly significant difference noted when comparing hexobarbital sleep times in animals treated only with saline (controls) and those treated with morphine (Table 2). Thus, the pituitary-adrenal axis must be intact to produce the characteristic decrease in drug response seen in the stressed animal, whereas the ultimate mediator of the response appears to be the adrenal cortex.

Smith *et al.* (2) have recently shown that the characteristic ascorbic-acid depletion of the adrenals and the elevation of blood corticosterone indicative of the stress response are present in the rat whose adrenal medulla has been

Table 1. Stress effects on duration of drug response. The drugs were administered after 2.5 hours of hind-leg ligation. The figures in parentheses are the number of rats in the group tested.

Adrenal ascorbic acid (mg% \pm S.E.)	Duration of drug response (minutes \pm S.E.)			
	Hexobarbital	Meprobamate	Pentobarbital	Barbital
787 \pm 43 (5)	24 \pm 1.1 (10)	Controls 129 \pm 3.7 (7)	73 \pm 2.7 (7)	221 \pm 18 (8)
		Stressed 102 \pm 1.8 (7)	50 \pm 1.8 (8)	266 \pm 22 (8)
490 \pm 26 (5) $p < .0005$	17 \pm 0.95 (8) $p < .0005$	102 \pm 1.8 (7) $p < .0005$	50 \pm 1.8 (8) $p < .0005$	266 \pm 22 (8) $p > .05$