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Identification of Chromosomes that Condition Dhurrin Content in Sorghum Seedlings¹

H. J. Gorz, F. A. Haskins, R. Morris, and B. E. Johnson²

ABSTRACT

Young plants of sorghum and sudangrass [*Sorghum bicolor* (L.) Moench] may be toxic to livestock because of the presence of the cyanogenic glucoside, dhurrin [*p*-hydroxy-(*S*)-mandelonitrile- β -D-glucoside], in the forage. In the present study a set of 11 chromosomal reciprocal translocations in 'Combine 7078' grain sorghum, involving each of the 10 chromosome pairs of sorghum in at least two of the translocations, was used to determine which chromosomes carried genes conditioning dhurrin content of sorghum seedlings. Each translocation stock was crossed and backcrossed once to a low-dhurrin line of sudangrass. Backcross progenies and parental plants were started in the greenhouse, and 100 plants of each backcross and 50 of each parental stock were transplanted to a field nursery in a randomized complete block design with five replications. Individual backcross plants were classified as semisterile or fertile on the basis of seed set. They were also classified for high or low dhurrin content based on a spectrophotometric assay of first leaves of 1-week-old seedlings grown from selfed or open-pollinated seed that had been harvested from the backcross plants. Results suggested the presence of one or more genes conditioning dhurrin content on at least 5 of the 10 chromosome pairs.

Additional index words: Cyanogenesis, Hydrocyanic acid, *p*-Hydroxybenzaldehyde, Prussic acid, *Sorghum bicolor* (L.) Moench, Spectrophotometric assay, Reciprocal translocations, Sudangrass.

THE cyanogenic glucoside, dhurrin [*p*-hydroxy-(*S*)-mandelonitrile- β -D-glucoside], is found in young plants of sorghum and sudangrass [*Sorghum bicolor* (L.) Moench] and its presence may make the forage from these plants toxic to livestock. Studies of the inheritance of dhurrin content in sorghum and sudangrass have resulted in variable and often conflicting conclusions. There is general agreement that dhurrin content, often expressed as hydrocyanic acid potential (HCN-p), is a heritable trait; but reports do not agree on such matters as dominance of high or low dhurrin content and the number of genes involved. In a review by Nass (11) on the inheritance of cyanogenesis in sorghum, two of the cited reports indicated that only one or two genes were involved in determining HCN-p, but four other studies reported that the inheritance of HCN-p was more complex, with multiple genes being involved. Krauss (9), in work involving crosses among four sorghum cultivars differing in HCN-p, concluded that HCN-p was governed by four gene pairs with additive effects and no dominance. In a recent study involving crosses of sorghum and low-HCN-p sudangrass, Lamb (10) found the inheritance of seedling HCN-p to be multigenic and complex, with additive genetic effects being the most important. The conclusion concerning the multigenic inheritance of dhurrin content in sorghum is reasonable when considered in

conjunction with extensive biochemical information that has been reported on the biosynthesis of dhurrin in sorghum (4). These studies demonstrated that dhurrin biosynthesis involved a series of enzymes in stepwise reactions, and it is reasonable to expect that a separate gene system controls each enzyme. Furthermore, some of the precursors involved in dhurrin biosynthesis also participate in other metabolic pathways. It would be expected, therefore, that many different genetic and metabolic alterations might ultimately affect dhurrin content.

Several workers (3, 5, 12) have studied relationships between visible plant characteristics and dhurrin content, but no reports were found in which genes influencing dhurrin content could be associated with specific chromosomes. A procedure for the identification of chromosomes carrying genes for specific traits using chromosomal reciprocal translocations was described by Anderson (1). In an individual that is heterozygous for a reciprocal translocation, the two translocated chromosomes pair with the two normals during meiosis in a cross-shaped configuration. At anaphase, when centromeres of homologous chromosomes go to opposite poles, the resultant gametes have a complete complement of chromosomes and are viable only when alternate chromosomes in the cross-shaped configuration move to the same pole. When adjacent chromosomes move to the same pole, chromosomal duplications and deficiencies result in nonfunctional gametes. Each of these events is expected to occur with a frequency of about 0.5. Thus, the seed set obtained on a translocation heterozygote is usually around 50% and the plant is said to be semisterile.

When a translocation heterozygote is backcrossed to a normal plant, half of the offspring would be expected to have full seed set and the other half (those carrying the translocation) would be semisterile. In anticipating expected results in our study, let us assume that an allele for high dhurrin content existed near the breakage point on one of the translocated chromosomes of a specific translocation stock, whereas the corresponding allele for low dhurrin content was carried by the normal parent used in crossing to that translocation stock. Then, in the backcross progeny the association of high dhurrin with semisterility and low dhurrin with normal seed set (these are the parental or noncrossover progeny) should be more frequent than the opposite association (nonparental or crossover progeny). Absence of a significant difference between the parental and nonparental associations would indicate that the gene under consideration was not located on the chromosomes involved in that translocation, or at least was not close enough to the breakage point to be detected.

The objective of this study was to identify, through the use of reciprocal translocations, chromosomes of sorghum that carry one or more genes conditioning detectable changes in the dhurrin content of seedlings.

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MATERIALS AND METHODS

The set of homozygous translocation stocks used was developed and furnished by K.F. Schertz (USDA-ARS and Texas A&M University) (13). The 11 stocks, isolated in progenies from irradiated pollen or seed of 'Combine 7078' grain sorghum, were high in seedling HCN-p (approximately 1000 mg kg⁻¹ fresh wt) and represented at least two translocations involving each of the 10 chromosome pairs of sorghum (Table 1). Each stock was hand-emasculated and crossed to an experimental low-HCN-p (approximately 350 mg kg⁻¹ fresh wt) inbred line of sudangrass (designated 78-415). The F₁s were backcrossed to another low-HCN-p (approximately 350 mg kg⁻¹ fresh wt) inbred line of sudangrass (designated 80-59) that was closely related to the parental low-HCN-p line but also was segregating for the *Ms₃/ms₃* gene for genetic male sterility. Male-sterile (*ms₃ms₃*) segregates in the 80-59 line served as females and the F₁s (*Ms₃ Ms₃*) as males in making the backcrosses. The *Ms₃/ms₃* gene for genetic male sterility was not involved in the semisterility associated with the reciprocal translocations, but did permit backcrossing without hand emasculatation and resulted in larger quantities of backcross seed than would have been obtained by hand emasculatation. All backcross plants were *Ms₃ ms₃* (male fertile); thus, these plants could be self-pollinated.

Backcross and parental seeds were planted in the greenhouse in April 1983, and seedlings were transplanted to rows spaced 0.76 m apart with a 0.61-m spacing within rows at the University of Nebraska Agronomy Farm, Lincoln, NE. The experiment consisted of five replications, each of which included 24 entries planted in 35 10-plant rows. Each of the 11 backcross entries was represented by two 10-plant rows per replication; each of the 11 homozygous parental translocation stocks of sorghum and the two parental low-HCN-p inbred lines of sudangrass was represented by one 10-plant row per replication. All backcross plants and a random sample of the parental plants were self-pollinated. Individual backcross plants were classified in the field as semisterile or fertile on the basis of seed set. Semisteriles generally had a seed set of about 50% while the seed set of fertiles was 90% or more; thus, the two classes were readily distinguishable. Self-pollinated (if available) or open-pollinated seed was harvested from each plant.

Plantings of selfed seed from equal numbers of semisterile and fertile backcross plants derived from the same translocation stock were made in growth chambers during March and April 1985 for assay of the HCN-p of a bulk of 10 seedlings from each entry. Selfed seed from the backcross plants,

rather than the backcross plants themselves, was used for the HCN-p assays so that the value for each backcross plant could be based on a bulk of 10 seedlings rather than on a single seedling. Most fully fertile backcross plants carrying a low-dhurrin allele near the breakage point of a translocation would be expected to be homozygous for that allele; the 10-seedling sample from such a plant should be relatively low in HCN-p. Most semisterile backcross plants, on the other hand, would be expected to be heterozygous for an allele located near the breakage point of the translocation. The 10-seedling sample from such a self-pollinated plant might include high-, intermediate-, and low-dhurrin individuals, and the HCN-p of the bulked sample should be higher than that of a homozygous low-dhurrin sample.

Each entry was represented in five replications, but the number of semisterile/fertile pairs within an entry was not the same in each replication. From 100 plants (20 in each of five replications) of each backcross that had been transplanted to the field, seed was harvested from a low of 73 plants to a high of 89 plants. Among these, HCN-p assays of semisterile/fertile pairs involved a low of 58 backcross plants (29 pairs) to a high of 84 plants (42 pairs) per translocation stock.

The growth chamber plantings were made in a soil mixture, and the chambers were operated at 27°C with continuous cool-white fluorescent light at a flux density of approximately 150 μmol m⁻² s⁻¹, as previously described (7). A bulked sample of 10 first leaves from 1-week-old seedlings was assayed for HCN-p by the spectrophotometric procedure of Gorz et al. (6).

Separation of individual high- and low-HCN-p backcross plants within the semisterile and fertile progeny of a single translocation stock was accomplished by calculating an entry mean within each replication; HCN-p values above the mean were designated high HCN-p, those below the mean value were designated low HCN-p. In effect, this procedure corrects the HCN-p classification for variation among replication means. Classifications for semisterility or fertility and high or low HCN-p were recorded for each backcross plant in each of the five replications, and frequencies of each of the four phenotypic classes were summed over replications. If HCN-p and degree of fertility were independent traits, equal frequencies of the four classes would be expected. A chi-square test for independence was calculated for segregations observed in the backcross progeny from each translocation stock, using the formula found in Strickberger (15) as follows:

$$X^2 = \frac{(ad - bc) - 1/2N)^2 N}{(a + b)(a + c)(c + d)(b + d)}$$

In this formula, *a* and *d* are the frequencies of the parental genotypes (semisterile, high HCN-p and fertile, low HCN-p) while *b* and *c* are the frequencies of the crossover or non-parental genotypes (semisterile, low HCN-p and fertile, high HCN-p). The *N* variable represents the total number of plants of the four types from one translocation stock and the two vertical lines in the numerator indicate the absolute or positive value of the difference within the lines. Chi-square values calculated in this way have 1 df. Equal frequencies of parental and crossover types would result in nonsignificant chi-square values, and as suggested in the introduction, would indicate that the chromosomes involved in that reciprocal translocation did not carry a major gene for HCN-p that was close to the breakage point.

RESULTS AND DISCUSSION

The data in Table 1 show the translocation stocks used, designations of chromosomes involved in the translocations, and segregations of high- and low-HCN-

Table 1. Sorghum translocation stocks, chromosome designations, segregations observed in backcross progenies from each translocation stock, and chi-square values for tests for independence.

Translocation stock	Chromosome designation	No. of backcross plants				χ ² value
		Semisterile		Fertile		
		High†	Low‡	High†	Low‡	
T-1	DA	23	9	12	20	6.31*
T-10	CB	21	8	7	22	11.67**
T-15	AE	25	17	14	28	4.79*
T-19	JD	22	20	17	25	0.77
T-24	FJ	26	12	9	29	13.56**
T-25	DG	24	13	10	27	9.20**
T-26	EF	29	9	7	31	23.28**
T-28	BH	27	13	9	31	14.60**
T-29	GI	28	9	11	26	13.88**
T-33	IC	28	8	7	29	22.24**
T-42	HF	20	16	11	25	3.63

*,** Denote significance at *P* ≤ 0.05 and 0.01, respectively.

† HCN-p greater than the entry mean.

‡ HCN-p lower than the entry mean.

p seedlings within the semisterile and fertile backcross progenies of low-HCN-p sudangrass \times (translocation stock \times low-HCN-p sudangrass). Significant or highly significant chi-square values were obtained for segregations from each of the translocation stocks except T-19 and T-42, indicating the presence of a gene(s) conditioning HCN-p in either one or both of the chromosomes involved in the other nine translocations. The inadequacy of karyotype analysis in sorghum at the time the translocation stocks used in these studies were developed does not permit more than the identification of the chromosomes involved. Identification of specific chromosome arms or portions of arms was not possible in this study.

In order to determine which of the two chromosomes involved in a translocation contained a gene for HCN-p in backcrosses resulting in a significant chi-square value, an evaluation was made of the segregations obtained in a second cross (and in some cases, a third cross) involving the same chromosome but interchanged with a different chromosome. By a series of comparisons of segregations in backcrosses involving different translocation stocks, chromosomes were classified as relatively effective or relatively ineffective in influencing HCN-p, but none of the chromosomes could be said with absolute certainty to have no effect on HCN-p. In this classification, an effort was made to minimize the number of chromosomes classified as effective.

The stepwise process in the classification of the chromosomes was carried out as follows (letters designate chromosomes, the T designation refers to the translocation stock used, * or ** indicates significance of the chi-square value at the 0.05 and 0.01 level, respectively, and NS indicates a nonsignificant chi-square value): JD(T-19) = NS provides a tentative indication that both J and D are ineffective. Results from FJ(T-24) = ** may be explained by assuming that F is effective. The observation that EF(T-16) = ** also is consistent with the hypothesis that F is effective. The chi-square value for HF(T-42) may have been nonsignificant because of the distance of the chromosome break in the HF translocation stock from the gene(s) influencing HCN-p in the F chromosome. If D is ineffective, as suggested above, then DG(T-25) = ** indicates that G is effective, and G, if effective, can account for GI(T-29) = **, allowing I to be classified as ineffective. If I is ineffective, then C must be effective to account for IC(T-33) = **. Inasmuch as H appears to be ineffective [HF(T-42) = NS], an effective B may be responsible for BH(T-28) = **. If both B and C are effective, CB(T-10) = ** is not surprising. DA(T-1) = * and AE(T-15) = *, combined with previously mentioned observations, suggest that A is effective and D and E are ineffective.

The foregoing analysis suggests that one or more genes conditioning HCN-p are located on chromosomes A, B, C, F, and G. This is the smallest number of chromosomes that can be considered to produce the results presented in Table 1. Following the same line of reasoning, chromosomes D, E, H, I, and J can be considered to contain no genes with significant effects on HCN-p. We recognize that chromosomes C and I could be switched between the two groups. That is, an effective I and an ineffective C, rather than an

effective C and an ineffective I, would also explain the observed results. However, the minimum number of effective chromosomes would still be five. Thus, the results obtained in these experiments suggest the presence of a minimum of five allelic pairs conditioning seedling HCN-p in the translocation stocks and sudangrass lines used in these experiments.

These results are consistent with previous studies (2, 3, 8, 10) in which it was concluded that the inheritance of HCN-p in sorghum was multigenic. Even more than five allelic pairs may be involved because some of the limitations in the use of chromosomal translocations for determining the number of genes conditioning a character, as described by Scott et al. (14), also would apply in the present study. For example, closely linked genes would probably be identified as a single gene, and unless a gene had a significant effect in the heterozygous condition, it would not be detected. Similarly, the use of translocation stocks to determine the association of chromosomes or portions of chromosomes carrying qualitative genes with a high degree of dominance is a powerful procedure, but it becomes less powerful when large numbers of genes are required for the full expression of a particular trait.

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