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WHEAT PRODUCTIVITY IMPROVEMENT IN THE DROUGHT PRONE AREAS OF KENYA

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ABSTRACT

In search for superior wheat (*Triticum aestivum* L.) varieties for the marginal areas of Kenya various breeding methods have been applied. These include introductions, mutation and the doubled haploid technique.The application of doubled haploid (DH) technique in breeding for drought tolerance has proved to be very effective and efficient for such complex characters as drought. This study was aimed at evaluating and validating doubled haploid lines (DHs) that were developed in 2000 using the chromosome elimination technique. The resulting lines have also been compared with other lines introduced from CIMMYT and two mutants developed in Njoro. The lines were tested in multi-locational trials in 2002 and repeated in 2003. The sites were Lanet, Mogotio, Naivasha, Mweiga, Kajiado and Katumani. The results show that the DHs were as good as the conventionally developed lines and had a better performance in yield than the check variety Chozi and Duma. One of the DHs had average yield of 1.7 t ha^{-1}, which was not significantly different from Chozi with the same mean yield and R960 with a mean yield of 2.0 t ha^{-1}. The DH technique therefore saves time of breeding without compromising quality of the output.

Key Words: Conventional breeding, drought tolerance, double haploids, mutation

RÉSUMÉ

Diverses méthodes ont été appliquées dans le but de trouver des variétés supérieures de blé *(Tricicum aestivum* L.*)* pour les régions marginales du Kenya. Ces méthodes comprennent les introductions, la mutation et la technique d'haploïde doublé. L'application de la technique haploïde doublé en matière de tolérance de l a sécheresse s'est avérée être très efficace et effective pour des caractères aussi complexes que la sécheresse. Cette méthode était destinée à l'évaluation et à la validation des haploïdes doublés (HDs) qui étaient développés en 2000 en utilisant la technique d'élimination chromosomique. Les lignées résultantes ont également été comparées à d'autres lignées introduites à partir de CIMMYT & 2 mutants développés à Njoro. Les lignées étaient testées dans des essais sur multiples localisations en 2002 et répétés en 2003. Les sites étaient Lanet, Mogotio, Naivasha Mweiga, Kajiado et Katumani. Les résultats montrent que les DHs étaient aussi bons que les lignées développées de manière conventionnelle et présentaient une meilleure performance au niveau du rendement par rapport aux variétés-contrôle Chozi et Duma. Un des DHJs avait un rendement moyen de 2.0 t ha^{-1}. La technique DH épargne donc le temps de culture sans toutefois compromettre la qualité du produit.

Mots Clés: Culture conventionnelle, tolérance de la sécheresse, haploides doubles, mutation

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the second most important cereal crop in Kenya (FAO, 2002) but Kenya's current national wheat production (approximately 300,000 tons per annum) meets only about 50% of the national demand. Moreover, the high increase in population and changing eating habits is expected to substantially increase wheat demand which is estimated to reach 850,000 tons p.a. in the year 2020 (FAO, 2003). Land area currently devoted to wheat production in the high potential areas is less than 2% and hence expansion in wheat production in these regions is limited. Horizontal expansion of wheat production has occurred (in recent years) in marginal rainfall areas (such as lower Narok, Naivasha, Laikipia and Machakos) which were hitherto considered unsuitable for wheat growing. Introduction of wheat in the non-traditional areas was started in 1992. Over this period 4 varieties (Duma, Ngamia, Chozi and Njoro BW1) have been released (Kinyua *et al*, 1989). More varieties with greater yields are still needed to meet the farmers' needs.

However, future production increases must come largely from vertical expansion (i.e., greater production per unit area), which will require more intensive research for further improvement of cultivars for yield potential and enhancing cultural technology. Wheat improvement in Kenya has been directed into developing broadly adapted, high yielding germplasm with high yield stability, durable disease resistance and acceptable end use quality. Resistance to biotic stress and tolerance to abiotic stress can be critically important to maintain high yield and thus contribute significantly to the adaptation of a given variety over time and location. Over the last decade wheat breeding has been enhanced by the application of various biotechnological approaches; these have been used to accelerate the breeding process and also complement the conventional breeding methods. The doubled haploid (DH) technique and mutation breeding have been used in breeding for various stresses in wheat. These include breeding for drought tolerance, tolerance to soil acidity, lodging tolerance and resistance to Russian wheat aphid.

Drought is a multidimensional stress affecting plants at various levels of their organisation (Blum, 1996). Therefore improvement of yield under stress must combine a reasonably high yield potential with specific plant factors which would buffer yield against severe reduction due to drought (Blum, 1996). Measurement of plant response to drought at the whole-plant level is complex because it reflects the integration of stress effects and responses at all underlying levels of organisation, over space and time (Blum, 1996). Conventional breeding (which involves multi-location testing and hence gauges spatial adaptation of genotypes) is used extensively by the Kenya bread wheat programme to identify temporally stable, drought tolerant germplasm. However, practical breeding programme for self-pollinated crops (such as wheat) must include a process of genetic fixation (for uniformity of agronomic traits) after genetic recombination to increase variability (Inagaki, 1996). Repeated selection of heterozygous material can increase uniformity but many generation cycles are required to reach homozygosity in loci associated with agronomic traits. The haploid production followed by chromosome doubling offers a quick method for developing homozygous breeding lines (Bentolila *et al.*, 1992; Murignaux *et al* 1993; Beanziger *et al.*, 1989; Baenziger, 1996). The doubled haploids (DH) derived from hybrid progenies can be used as recombinant inbred lines with favourable gene combinations (Inagaki, 1996). This technique could thus complement the conventional breeding programmes to accelerate the release of new varieties.

Since the double haploids are completely homozygous, all stocks are identical and no purification process is required. In contrast, in the conventional system stocks are usually derived from a single plant of an advanced generation (Baenziger, 1996) hence several generations are required to build up sufficient quantities of seeds for release. Compared to selection during the early generation of a pedigree, a DH system increases the efficiency of selection for qualitative, major gene characters and in particular for quantitative characters. Therefore, this study was aimed at evaluating and validating doubled haploid

lines and consequently compare the technique with conventionally screened lines. It was hypothesised that the DH technique is as superior to conventional breeding especially while dealing with complex factors like drought.

MATERIALS AND METHODS

Two experiments were carried out. One of which involved development of the double haploids (including embryo rescue and regeneration of haploid plantlets, chromosome doubling of haploids) and preliminary evaluation of the DHs. While the other was a field evaluation of the performance of the DHs as compared with the conventionally developed lines.

Development of doubled haploids. Six F_1 hybrids were produced by crossing three drought tolerant lines (Duma, K. Mbweha and Ngamia) as female with two high yielding commercial varieties (Kwale and Kenya Chiriku) as males. The F_1 plants were emasculated at anthesis. The glumes were not clipped with the awns, as is the case with normal-crossing emasculation process. The emasculated spikes were then covered with polyethylene bags to maintain high humidity. One day before (predicted) anthesis, emasculated spikes were pollinated with freshly collected maize pollen. The pollen was collected by picking mature anthers and placing on petri-dishes. Once the anthers burst to release the pollen, a soft brush was used to brush the emasculated spikelets with the pollen. Extreme care was taken not to damage the stigma. After pollination, the uppermost internodes of wheat culms (with pollinated spikes) were injected with a 100 mg l^{-1} 2, 4-D solution daily (for two consecutive days) to increase the rate of fertilisation and embryo formation.

After about 14 days of spike growth, wheat caryopses were removed from spikes and sterilised (in 2% sodium hypochlorite) for 15 minutes. After rinsing with sterilized water (under laminar flow bench), embryos were aseptically excised and transferred onto half strength Murashige and Skoog medium supplemented with 20g L^{-1} sucrose and 8g L^{-1} agarose in petri dishes (Inagaki, 1996). These were then placed in the fridge (at 4 °C) for three days after which they were incubated (at 25° C) in the dark until embryos germinated (5-7 days). The germinated embryos were transferred to a lighted growth room with controlled temperature of 24 °C and 16 hours day length. The light intensity was Ca 5000 Lax. Cultured plants with fully developed roots and leaves were transplanted to potted soil for further growth. The temperature was maintained at 21 °C -25 °C using 24 hours day length. The size of the stomata was used to determine the ploidy level of the plants. This is because haploid plants have stomata half the size of diploid plants. At the third tillering stage, a leaf from each plant was cut and coated with clear nail varnish. The varnish was pealed off (upon drying) and mounted on high power objective of light microscope; the size of the stomata was then compared with that of known diploid plants.

Also, at the third tillering stage the plants were watered and then removed from the pots. The roots were trimmed to about 2 cm below the crown (so as to increase solution absorption). The plants were then immersed in colchicine solution (0.2% colchicine, 2% dimethyl sulfoxide and 15 drops of tween-20) for 3 hours at room temperature. The plants were then washed thoroughly with running tap water for 3 hours after which they were planted in pots (using forest soil enriched with copper dust) at a temperature of 20° C-25 ° C under high humidity (90-100%).

Preliminary evaluation of the doubled haploids. Twenty DH lines were selected (based on the amount of seeds available). These selections and their five parents were initially screened in the rain-shelter at National Plant Breeding Research Centre (NPBRC) Njoro in 2000 and later evaluated in the field (in Njoro and Katumani) through observation trials in 2001. They were planted in a randomized complete block design (RCBD) with three replications. Each plot was 1 metre long with two rows 20 cm apart. Five seeds were planted per row. DAP (18-46-0) fertiliser was applied at planting at the rate of 150 kg ha^{-1}. Drip irrigation was used to water the plots. Water was applied at the rate of 20 mm every fortnight (up to grain filling stage), giving a total of 200 mm water during the crop season. The amount and frequency of water application simulates the amount and

nature of rainfall pattern usually received in most marginal areas during cropping season (Jaetzold and Smith, 1983).

The following parameters were measured (both in the rain shelter and in the field): ear length (measured from the base of the spike to the tip of the apical spikelet, excluding the awns); number of spikes per plants, sterile spikelets per head; number of grains per head counted on 10 spikes selected randomly in each experimental unit at maturity; and weight of 10-kernel weight (g). All the data was subjected to analysis of variance using the general linear model.

Field performance of the double haploids. Eight DH lines (DH4, DH5, DH6, DH7, DH9, DH12, DH15, DH16) and 2 mutants (BM1 and BM3) were selected in a preliminary yield trial in 2001. The selection was on the basis of drought tolerance. These lines, together with Chozi and Njoro BW1 (checks), were entered in the National Performance Trial (NPT) in 2002 and consequently planted at three sites (i.e., Naivasha, Katumani, and Lanet). The design was an RCBD replicated 3 times. The seeds were drilled in plots that consisted of four rows that were 6 metres long and 20 cm apart.

Seven DHs (DH4, DH5, DH6, DH7, DH9, DH12 and DH16) were selected form the first year of NPT and entered into the second year of National Performance Trial (in 2003) alongside 7 conventionally developed lines (R840, R960, R962, R963, R965, R966 and K7872) and the check varieties (Njoro BW1 and Chozi). These were evaluated at Katumani, Naivasha, Mweiga, Lanet and Ravine. The Design was a RCBD replicated three times. The plots were 4 rows that were 20 cm apart and 6 m long.

The following parameters were measured in both the first and second year NPT: yield, kernel weight, plant height and reaction to rust diseases. ANOVA was used for data analysis and means were separated using LSD.

RESULTS AND DISCUSSIONS

Development of doubled haploids. A total of 1800 florets were cross pollinated out of which 890 F_1 seeds were harvested and the embryos exised and planted *in-vitro*. This shortened the F_1 seed production by over four months compared to the *in-vivo* method where the seed is left to dry in the field. The *in-vitro* F_1 plants grew faster due to the conducive environment and reached heading stage one month earlier than in the conventional method.

Over 2,880 florets of F_1 plants were cross pollinated with maize of which 413 developed seeds and 57 embryos were rescued (data not presented). Out of the 57 embryos rescued 46 were haploid. When treated with colchicine 24 of the 40 survived. The time taken for pollination to colchicine treatment was 8 weeks and the DHs were ready for harvesting in 20 weeks.

Performance of doubled haploids. There was high variability between the DHs in the rainout shelter for the yield components that were measured (Table 1). These results are comparable to those reported by Baenziger (1996) that there may be variation among doubled haploid lines but there is little variation within an individual haploid line. This increased the efficiency of selecting lines with superior characteristics (Njau *et al.*, 2000) because the response to selection is indicated by the variability between the treatments. Such variation can be used to pick the lines with the required characteristics (Njau, 2001).

There was significant difference in yield and other growth parameters between the DHs and their respective parents (Table 1). This was an indication of variation created. Also, some DHs showed heterosis for drought tolerance over their better parent or the mean of the two parents (Table 1). For example, one of the DHs ($DH_1$7) developed from the cross between Kenya Mbweha and Kenya Chiriku had greater number of spikes (6.1) than the parents (2.0 and 5.0, respectively for Kenya Chiriku and Kenya Mbweha). Also, $DH_1$7 had more grains per head (43) than the mean of the parents (27.5). $DH_1$12 (developed from Duma/ Kenya Chiriku cross) had more grains (%) than either of the parents. The expression of heterosis in DH lines (Njau, 2002) makes the DH technique superior to that of conventional breeding. This is because heterosis is lost in conventional breeding due to repeated selfing of the hybrids. Similar results have been reported by Ba-Bong and Swaminathan (1995).

TABLE 1. Mean performance for various parameters of 20 DHs as compared to their parents tested for drought tolerance under the rainshelter at Njoro. Kenya

Parents	DH lines	No. of tillers at booting	Effective heads	Spikelets /head	Sterile spikelets	Grains/ head	Weight of 10 grains		
K. Mbweha		5.00	5.0	15.533	2.000	24.333	0.367	Kwale	
	4.21	2.16	14.533	3.994	27.936	0.306	(K.mbweha	$DH_1$1	5.33
	4.00–	16.000**	2.00*	34.00	0.301				
x Kwale)	$DH_1$8	5.71	3.67–	17.533**	2.494*	35.936	0.317**		$DH_1$19
	3.71– –	3.66	15.333	1.494*	32.936	0.27–	K. Chiriku		4.33
	2.00	15.333	3.000	30.667	0.312				
(K. Chiriku x	$DH_1$7	7.39**	6.11**	19.301	2.009	43.489***	0.282		
K Mbeha)	$DH_1$14	2.67– –	2.33	17.333	1.333*	36.667**	0.343		
Ngamia		3.67	3.00	10.00	1.667*	41.667	0.349		
(Ngamia x Kwale)	$DH_1$2	4.39	4.11	19.301**	1.009*	41.489**	0.336		$DH_1$3
	5.21	3.66	16.033**	1.995*	24.94–	0.21–			
	$DH_1$10	4.33	2.00–	13.333	1.006*	27.67–	0.346		
	$DH_1$13	4.30	3.95	15.349**	1.996*	41.755**	0.304		$DH_1$15
	3.33–	3.33	15.333**	0.67***	30.00*	0.367	$DH_1$16	8.39**	6.1***
			19.301**	0.09***	28.489	0.304			
(Ngamia x	$DH_1$17	0.803– –	0.45—	13.765	4.980—	4.33—	0.12—		
K.Chiriku)	$DH_1$20	5.00	4.33	14.000**	1.667**	28.67–	0.19– –		
Duma		6.33	3.00	15.000	2.000	28.333	0.467		$DH_1$4
	2.80– –	2.45	13.349	2.996	19.255	0.305–	(Duma x Kwale)	$DH_1$5	
	3.39	3.11	19.301	2.009**	35.489	0.310–	$DH_1$6	7.39	2.89
	14.617	1.510**	33.308	0.367–		$DH_1$9	7.00	4.00	11.333
	2.333**	29.333	0.266–		$DH_1$18	4.30	2.45	12.349	1.996**
	32.255	0.326–							
(Duma x	$DH_1$11	3.71–	2.16	14.533	0.995	40.936*	0.307*		
K.Chiriku)	$DH_1$12	5.67	3.67	17.333	2.000	44.00**	0.421**		

* Significantly better at 5% level than the poorer parent; ** Significantly better than the mean of the parent at $P \leq 0.5$; *** Significantly better than the better parent at 5 % level.

Over 26% of the cost was saved in the production of the DHs (data not provided). This saving was attributed to the fewer number of generations required to produce homozygous lines when using haploids. Similar findings have been reported in rice (Sunint, 1993).

Preliminary performance of the double haploids. Significant differences in yield were noted in Katumani and Naivasha while no differences were noted in Lanet (Table 2). Two lines DH6 and DH4 performed quite well in all the sites. DH6 was better than all the other entries in Naivasha and it was better than the Checks in all the sites. DH7 and DH9 also did well in other sites (data not presented).

Genotype did not affect 1000-kernel weight in Katumani and Naivasha but genotype affected 1000-kernel weight in Lanet (Table 3). The double haploids had greater 1000-kernel weight (by 16% on average) than the check varieties and the mutant BM3 (by 8% - 19%; average 12%) than) in Lanet (Table 3). Also, the mutant BM1 had 9% - 13% greater 1000-kernel weight than the double haploids in Lanet (Table 3).

Genotype affected the average coefficient of infection (ACI) by stem rust in 2002 (Fig. 1). Chozi (check variety) was more susceptible to stem rust than the double haploids (except DH15 and DH16) and the mutants (Fig. 1). NjoroBW1 (check variety) had lower stem rust infection than the mutants and most double haploids (DH5, DH9, DH12, DH15, DH16) (Fig. 1).

Performance of the DHs as compared to the conventionally developed lines. There was a significant effect of variety due to site differences in terms of both grain yield and hectolitre weight in 2003 (Table 4). Katumani had lower yields than Lanet (by 80%), Mogotio (by 70%), Naivasha (by 70%), Mweiga (by 30%) and Kajiado (by 30%). Also, Kajiado and Mweiga had lower yields compared with Lanet (38%), Mogotio (31%) and Naivasha (31%) (Table 4). The lower yield in Katumani (compared with other sites) could be due to the low average rainfall (100 mm) recorded in Katumani during the growing period of the crop.

Lanet had lower (by 20%, 19%, 10%, 13% and 7% for Mogotio, Naivasha, Mweiga, Kajiado and Katumani, respectively) hectolitre weight compared with the other sites (Table 4). Katumani had 12%, 11% and 5% lower hectolitre weight than Mogotio, Naivasha and Kajiado, respectively.

TABLE 2. Grain yield of the test lines (double hybrids, mutants and check varieties) at 3 different sites (t ha^{-1})

Line	Yield (t ha^{-1})		
	Katumani	Naivasha	Lanet
DH4[2]	0.9	1.0	1.4
DH5	0.3	.3	1.1
DH6	0.9	1.5	1.5
DH7	1.0	1.3	1.1
DH9	0.7	1.4	1.6
DH12	0.7	0.9	1.2
DH15	0.7	0.7	1.3
DH16	0.8	0.8	1.2
BM1	0.6	0.8	1.0
BM3	0.7	0.7	1.3
Njoro BW1[3]	0.8	0.9	1.3
Chozi[3]	0.5	0.9	1.1
LSD	0.27	0.37	0.56
SE		0.22	0.11
P(F-ratio)	<0.05	<0.05	<0.05

[1]Values followed by the same letter are not significantly different at P = 0.05
[2]DH = Double hybrid, BM = mutant
[3] = Checks

Also, Mweiga had lower hectolitre weight compared with Mogotio (9%), Naivasha (8%) and Kajiado (2%). Moreover, Kajiado had 6% lower hectolitre weight compared with Lanet and Mogotio (Table 4).

The yields and hectolitre weight for the lines averaged across the sites are shown in Table 5. Genotype affected the grain yield and hectolitre weight. Chozi (check variety) had 55% more yield than DH6 and DH16 and 113% greater yield compared with DH12. Also, NJOROBW1 (check variety) had higher yield (by 88%) compared with DH12 (Table 5).

Chozi had higher hectolitre weight compared with R960, R962 and R966 (9%), K7872 (12%), R965 (10%), which are lines developed through

TABLE 3. 1000 kernel weight of the entries in the three sites during the year 2002

Line	1000-Kernel weight (g)		
	Katumani	Naivasha	Lanet
DH4	76.6	73.4	83.1
DH5	72.0	78.8	81.0
DH6	72.4	76.7	78.7
DH7	72.4	72.4	78.4
DH9	70.7	71.2	78.0
DH12	77.4	78.3	78.0
DH15	75.9	75.8	77.9
DH16	76.6	76.4	77.4
BM1	73.9	72.7	75.6
BM3	72.9	68.2	69.7
Njoro BW1	74.2	76.1	69.4
Chozi	75.3	78.6	66.9
LSD	-	-	10.85
P (F-ratio)	ns	ns	<0.05
SED	4.1	3.2	4.04

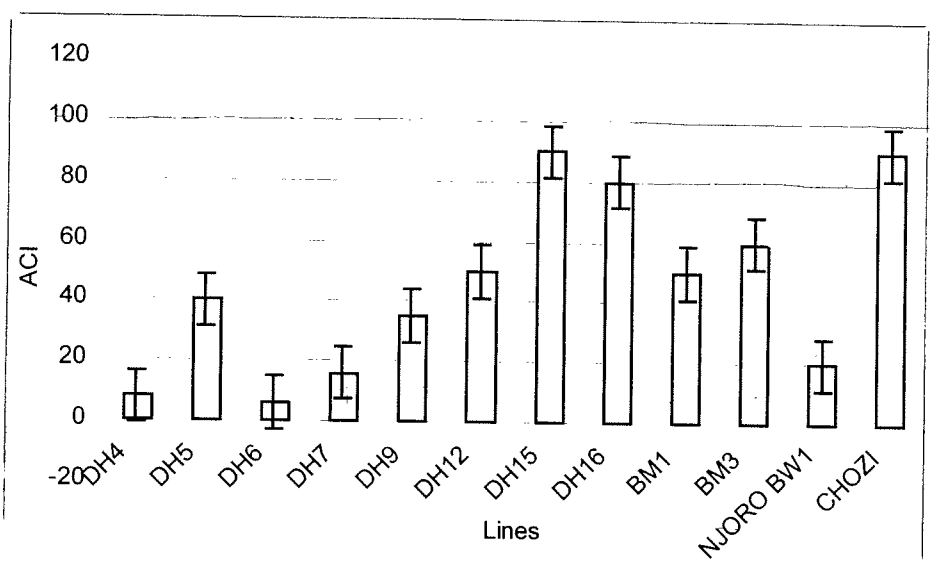

Figure 1. Stem rust average coefficient of infection.

TABLE 4. Average yield in tons per hectare and hectolitre weight in the six sites

Site	Yield (t ha^{-1})	Hectolitre weight (g)
Lanet	1.8a	67.3d
Mogotio	1.7a	80.9a
Naivasha	1.7a	80.2a
Mweiga	1.3b	74.3c
Kajiado	1.3b	76.0b
Katumani	1.0c	72.1c
Mean	1.5	75.1
LSD (0.05)	0.18	1.70
SED	0.21	4.21
P (F-ratio)	<0.01	<0.01
CV (%)	29.7	5.6

TABLE 5. Yield and hectolitre weight of the sixteen lines averaged over the sites

Line	Yield (t ha^{-1})	Hectolitre weight (g)
R960	1.9a	74.5bcd
DH4	1.7a	73.0cd
R840	1.7a	78.1ab
Chozi	1.7a	81.3a
R966	1.6ab	74.3bcd
R963	1.6ab	76.6abcd
K7872	1.5abc	72.3cd
DH9	1.5abc	75.0bcd
NJBW1	1.5abc	77.0abc
DH5	1.5abc	73.5bcd
R965	1.5abc	73.9bcd
DH7	1.4abc	74.7bcd
R962	1.4abc	74.6bcd
DH16	1.1bcd	72.9cd
DH6	1.1cd	78.0ab
DH12	0.8d	72.0d
Mean	1.5	75.1
LSD (0.05)	29	2.77
SED	0.09	17.7
P (F-ratio)	<0.01	0.01

conventional breeding (Table 5). Also, Chozi had greater (by 8%-13%; average 11%) hectolitre weight compared with a number of double haploid lines (DH4, DH9, DH5, DH7, DH16, DH12) (Table 5). Another check variety (NJOROBW1) had 7% greater hectolitre weight than DH12 (Table 5). These results show that in addition to saving time, the DH technique, compares well with the conventionally developed lines which have taken over 13 years to develop.

CONCLUSION

The DH technique proved to be quite effective in breeding for complex characteristics such as drought and compares well with the conventional methods. The time saved in DH development makes the method more superior to the other methods. It is important to note that the multiloctional testing increases the efficiency of measuring for stability. The two lines (DH4 and R960) are recommended for release they out yielded the check varieties in almost all the sites.

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