EMS has been amply demonstrated to be a good mutagen of nuclear genes. Hence it might be a good cytoplasmic mutagen if the genetic material is similar for both methods of inheritance. There are a few reports of the induction of cytoplasmic mutants in plants. Dulieu (3) used EMS to induce chlorophyll deficient mutations in Nicotiana that were maternally inherited. Favret and Ryan (4) have induced cytoplasmic male sterile mutants in barley with x-rays and with EMS. Also, Lysikov et al. (5) reported that cytoplasmic male sterility has been induced in maize by chemical and physical mutagens.

Failure to detect cytoplasmic male sterility in the EMS treated material may be due to relatively small populations used; also, cytoplasmic male sterility may occur at a very low frequency.

References

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Robert W. Briggs

DEFIANCE COLLEGE
Defiance, Ohio

1. The effect of red and far-red light interruptions on paramutant R expression.

In MGCNL Vol. 43 we reported that the level of aleurone pigment produced by R' (paramutated R) was directly related to the number of dark periods administered to seedlings at an early stage of tassel initiation. We have been concerned with defining more closely those periods of development when R' is most sensitive to genetic "instruction" as well as finding more effective treatments for making heritable changes in R' expression.

Since much physiological work over the past 20 years has indicated that many plant systems are responsive to red and far-red light, it was necessary to find ways of testing the paramutational system of $\underline{RR}^{st}$ for its responses to both red and far-red. This report is concerned with changes in $\underline{R}'$ expression following red and far-red light interruptions of tassel-inducing dark periods.

Inbred W22 seedlings, heterozygous for $\underline{RR}^{st}$, were sown at growth chamber temperatures of 26-27° C. under continuous light supplied by 14 200W cool white fluorescent lamps 80 cm. from the surface of the soil. At 12 days of age, seedlings were subjected to six cycles of 12 hours of light and 12 hours of darkness (12:12,L:D) for six successive days. Each dark period was interrupted for one hour with red, far-red or white light during the 3rd, 5th, 7th or 9th hours--making a total of 12 different treatments.

Red light was provided by filtering with one eighth inch red plastic (Rhom and Haas) with a transmission maximum between 650 and 675 nanometers. Far-red light was supplied by filtering with combined red and blue plastic where the blue filter showed a transmission maximum between 450 and 475 nanometers. The light source for the above filters was provided by one 150W incandescent bulb with built-in reflector. The bulb was positioned 60 cm. above the tops of the plants. White light interruption was supplied by two of the above incandescent bulbs 60 cm. from the tops of plants.

Following L:D treatments, plants were held in constant light until the 20th day at which time all seedlings were transplanted to field conditions for maturation. Testcrosses of each of the above series of treated plants were made on Inbred W22 tester, $\underline{rr}$, females. 50 $\underline{R}'$ kernels from each testcross ear were scored for aleurone pigment level by matching each against a set of standard kernels ranging from colorless, assigned a value of 0, to completely pigmented, assigned a value of 22. Kernel means for each testcross ear are reported along with treatment means and their variance. Results of t-test comparisons are reported.

Table 1 shows that treatment means for $\underline{R}'$ expression from seedlings which received red light interruptions at the 3rd and 5th hour of the dark period differ significantly from means of $\underline{R}'$ kernels

representing plants whose dark periods were interrupted with far-red either the 7th or the 9th hours ($P =< .01$). It can be noted that the R' scores of seedlings interrupted with white light lie between scores of those seedlings which received red and far-red light interruptions. Among those seedlings interrupted with red light, plants treated the 3rd hour of the dark period produced R' scores different from those treated the 7th or the 9th hours ($P =< .05$ and $P =< .02$, respectively).

Table 1

Comparison of R' expressions following light interruptions of dark period. Treatment means of R' kernels from testcrosses of RR^{st} plants which had six dark periods interrupted by one hour of light beginning at the designated times.

Color of Light Interruption	Hour of Dark Period for Light Interruption (each of six nights)			
	3	5	7	9
Red	n = 8	n = 10	n = 13	n = 9
	12.70	13.35	13.99	14.79
Variance	.80	1.64	2.03	3.88
Far-Red	n = 11	n = 10	n = 8	n = 8
	14.85	14.69	15.10	15.64
Variance	3.47	1.34	.71	.85
White	n = 9	n = 10	n = 11	n = 9
	13.75	13.97	14.76	13.45
Variance	1.35	1.07	1.79	3.09

Table 2

Ear means of R' expression from RR^{st} plants which received light interruptions of their dark periods.

These data are summarized in Table 1.

Color of Light Interruption	Hour of Dark Period for Light Interruption			
	3	5	7	9
Red	11.92	15.08	12.66	15.28
	11.29	14.69	14.90	13.36
	13.24	12.80	15.18	15.16
	13.70	14.82	15.53	17.66
	12.94	13.30	12.96	12.88
	12.22	12.82	14.70	16.40
	12.44	12.74	14.00	16.82
	13.86	11.26	13.46	11.80
		11.92	10.26	13.76
		14.02	13.84	
			14.38	
			15.04	
			14.96	
Far-Red	13.32	13.72	15.58	14.18
	16.92	14.96	14.53	16.72
	13.08	14.84	14.82	16.88
	16.02	13.42	14.98	15.02
	14.58	13.98	14.02	15.24
	11.68	13.94	15.00	15.24
	14.12	17.18	16.86	15.52
	15.82	15.82	14.98	16.30
	18.02	15.18		
	13.92	13.89		
	15.90			
White	15.56	14.59	13.10	16.69
	13.82	14.84	14.86	12.12
	13.88	15.86	15.37	10.62
	14.10	12.60	13.74	12.16
	11.56	13.54	13.64	13.28
	13.20	14.80	16.00	13.82
	14.18	13.66	16.46	14.00
	14.74	13.42	16.96	14.98
	12.71	12.70	15.00	13.39
		13.64	13.12	
			14.10	

A variance increase can be noted for plants which received red light interruptions toward the end of the dark period whereas score deviations are greatest for plants which received far-red interruptions early in the dark period. The data suggest red light, applied early in the dark period, has a greater probability of conditioning the R' expression to a lower level of expression in the W22 Inbred. Far-red has the opposite effect of red light and is most effective when applied toward the latter part of the dark period.

The difference in effect of red and far-red on R' expression is of that reported for various L:D treatments at early stages of development.

Bernard C. Mikula
Jordan Christiansen

2. Heritable differences in paramutant R expression after one growing season in different latitudes of the United States.

In previous reports, MGCNL Vols. 41, 42, 43, we have shown that heritable levels of R' (paramutated R) expression are conditioned by light-dark cycles applied early in development. It seemed likely, therefore, that different levels of R' expression could be conditioned by natural environments if RR^{st} plants were grown out one season at different latitudes. Such latitudinally induced heritable differences in gene expression would pose a very interesting challenge to existing biological dogma regarding sources of genetic variation for evolution. To test the hypothesis that one growing season in a different environment can make a heritable difference in a gene expression, a common lot of seed was divided and distributed to each of six locations across the latitude of the United States.

Seeds of Inbred W22 RR^{st}, from a cross involving a single pollen parent of RR to $R^{st}R^{st}$ female sib plants, were distributed to Wisconsin, Illinois, Iowa, Texas, and a final sample was grown in Ohio (a Missouri sample was lost because of adverse weather conditions). At each of the localities named above the Inbred W22 RR^{st} plants were testcrossed to Inbred W23 rr. The hybrid seed (W22 and W23) was then returned to Defiance, Ohio to be grown out and testcrossed to assay the level of R' expression.