of Dorman.

If the gene conditioning PMV resistance in Peking were the same as in the other three resistant lines, then one would not expect segregation in any  $F_2$  families from crosses among these lines. However, segregation was observed in all  $F_2$  families from crosses of Peking with the other resistant parents. Apparently, at least two different genes for resistance were present. The only two-class  $F_2$  dihybrid ratio that would provide a reasonable fit to the data is 13 resistant : 3 susceptible. This ratio is possible if one assumes that Peking has a recessive gene for resistance. As shown in Table 2, the data provide a very acceptable fit to that model. The susceptible reaction of the  $F_1$  plant from the cross Peking (R) x PI 229.315 (S) seems to further substantiate the hypothesis of a recessive gene for PMV resistance in Peking. The expected  $F_2$  genetic ratio from that cross would be 1 R : 3 S. Data are not yet available.

Based on the available data, it appears that PMV resistance in Peking is conditioned by a gene in the recessive state which is independent of the single dominant gene reported by Boerma and Kuhn. While both PI 89.784 and PI 219.789 contain genes which interact with the Peking gene in a similar manner as that from Arksoy, it remains to be shown that they contain the same dominant allele. Investigations on the allelic relationships of sources of PMV resistance are being continued.

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# 1) Cytology of soybean haploid progeny.

Haploids are being isolated annually among individuals obtained from polyembryonic seeds associated with the North Carolina male sterile  $(ms_1)$ .

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The haploids are being used to obtain aneuploids. In 1976 and 1977, 7,206 and 15,530 seeds, respectively, were obtained from male sterile plants ( $\underline{ms_1}$  North Carolina) representing Maturity Groups I-V. These seeds were germinated in the laboratory and screened for polyembryony in a search for soybean haploids (2n = 20). In 1976, five haploids were obtained from 167 polyembryonic seeds and one haploid was from a monoembryonic seed (Beversdorf and Bingham, 1977). In 1977, four haploids were obtained from 252 polyembryonic seeds. All monoembryonic progeny were screened phenotypically for haploidy; however, none was identified.

From the 1976 haploids, 45 seeds were obtained by either hand cross pollination with diploids or by about 10 apparent self pollinations. Most of these seeds germinated and progeny were grown in the greenhouse in 1977 and analyzed cytologically. The progeny consisted of 42 diploids, one triploid, one 70chromosome plant, nine tetraploids, and only two trisomics. Ten seeds were obtained from the four 1977 haploids. These progeny consisted of nine diploids and one putative trisomic.

Currently, we are analyzing  $F_2$  progeny from the trisomics for percent transmission. Palmer (1974) isolated several trisomics from asynaptic mutants and obtained a high percentage of trisomic progeny. Microsporocyte analysis of the  $F_1$ 's was incomplete due to a lack of suitable diagnostic stages; however, restitution gametes in various stages of cytokinesis were frequently noted in most of the  $F_1$ 's. Some spindle abnormalities including parallel spindles were also observed.

The predominantly euploid 2n = 40 progeny of haploid x diploid crosses suggest that the <u>ms</u> allele is functioning in these haploids to produce restitution gametes. This may occur in the male gametophyte by failure of cytokinesis as shown by Albertsen (1976), or in the female gametophyte by fusion of supernumerary nuclei found to be present at the time of fertilization (Cutter and Bingham, 1977).

A few trisomics are being obtained using haploids, but no monosomic or other deficiency aneuploids have yet been confirmed. The  $\underline{ms}_{1}$  gene, which is carried by the haploids and which is associated with restitution gametes, is likely limiting the yield of both excess and deficiency aneuploids.

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#### 2) Potential soybean vigor tests for screening at low germination temperatures.

Introduction: Littlejohns and Tanner (1976) have used soybean germination at low temperature (10°C) as a criterion for selecting for "cold tolerance". It is our contention that this coupled with a more definitive vigor test might prove to be more reliable in evaluating genotypic differences at low germination temperature. The most prominent soybean vigor tests have been classified into five major categories by McDonald (1976): (1) cold test, (2) accelerated aging test, (3) tetrazolium test, (4) respiration test, and (5) conductivity test. As McDonald has pointed out, most of these tests are somewhat subjective and have inherent problems associated with them. Our recent studies (Duke et al., 1977a; Duke et al., 1977b) have shown physiological and biochemical data which indicate that more quantitative methods may be used in testing soybean vigor, especially at low temperature (10°C). Our data relate to screening for cold tolerance and to all of the aforementioned vigor tests except the accelerated aging test, in that we have studied the effects of low temperature on soybean germination (cold test), mitochondrial respiration (respiration test), mitochondrial integrity (conductivity test), and dehydrogenases (tetrazolium test). In addition, we have studied the effects of low temperature on the production of asparagine, a major transport amino acid in soybeans, during germination. Presently it appears that three of the physiological and biochemical parameters mentioned here have potential as practical quantitative indicators of soybean vigor at low germination temperature.

<u>Mitochondrial integrity</u>: Glutamate dehydrogenase (GDH) may be used as an indicator of mitochondrial integrity because GDH is only located in mitochondria of etiolated plants (Duke and Ham, 1976). Our past studies have shown data similar to that in Table 1 which indicates that low temperature Table 1

Percentages of GDH recovered in soybean (cv. Wells) mitochondrial pellets (20,000 g) germinated at optimal and suboptimal temperatures

	2.6.1.4	2 days	5 days	
Axes	10°C	39.7%	44.1%	Pellets were solubilized by
	23°C	100.0%	100.0%	freeze-thawing 3 to 5 times. GDH was assayed as previously described (Duke <u>et al.</u> , 1975). Total GDH activities, from
Cotyledons	10°C	20.6%	26.8%	
	23°C	42.0%	38.2%	which percentages were calcu- lated, were by the addition of supernatant (20,000 g) and mitochondrial (20,000 g) values

has a great influence on mitochondrial integrity. When mitochondria are not fully developed, as in early stages of germination, their membranes are easily disturbed during extraction. Soluble enzymes, such as GDH, can then leak from mitochondria into mitochondrial extraction media. This may be what we are observing here: differences in stage of mitochondrial biogenesis. However, our previous study (Duke <u>et al</u>., 1977a) would suggest that membrane phase changes at low temperature could also account, in part, for differences in mitochondrial integrity.

From the data presented here it appears that axes values reflect the physiological states of mitochondria to a greater extent than cotyledon data. Presently we are conducting further tests to establish the validity of this test as an indicator of vigor in soybeans grown at low temperature.

<u>NADP-isocitrate dehydrogenase (NADP-ICDH) activity</u>: In a previous study (Duke <u>et al.</u>, 1977a) we determined that increases in NADP-ICDH activity reflect the onset of germination of soybeans. This enzyme increased in activity before any of the other dehydrogenases assayed during germination. This would indicate that it might be very important in energy transduction early in germination. Also, we found that kinetic data from mitochondria were similar to those of NADP-ICDH at low temperature, indicating that this enzyme might be limiting at low temperature in soybean germination. We are currently investigating this assay as a possible screening device for soybeans at low temperature.

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<u>Free asparagine</u>: Our previous study (Duke <u>et al.</u>, 1977b) has shown that asparagine is higher in concentration than any other amino acid during soybean germination. Low temperature was shown to inhibit the production of asparagine from its precursor, aspartate. Table 2 indicates that differences in asparagine at 10° and 23°C are greatest in axes tissues. The asparagine assay is more complicated than either the NADP-ICDH assay or the test for mitochondrial integrity. However, it appears to be more indicative of vigor than the other tests. Presently we are attempting to find a more practical assay for asparagine.

### Table 2

Concentrations of aspartate and glutamate and their amide derivatives, asparagine and glutamine, in soybeans (cv. Wells) germinated at optimal and suboptimal temperature for 2 days

	and wan we	23°C	10°C		
Tada	Axes	Cotyledons	Axes	Cotyledons	
asparagine	23.3	1000100 2.10 9961a 1	0.000	0.434	
aspartate	1.34	3.21	2.98	0.706	
glutamate	1.86	2.90	2.50	0.711	
glutamine	0.55	1.15	0.964	0.064	

\*Concentrations are in  $\mu$ moles g<sup>-1</sup> fresh wt., and were determined by TLC.

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