

TABLE I

No.	R <sub>1</sub>	R <sub>2</sub>	m.p. °C.	Empirical formula	Nitrogen Per cent	
					Found	Calculated
1	C <sub>6</sub> H <sub>5</sub> -	<i>m</i> -C <sub>6</sub> H <sub>4</sub> NO <sub>2</sub>	165	C <sub>15</sub> H <sub>11</sub> O <sub>2</sub> N <sub>3</sub> S	14.1	14.1
2	C <sub>6</sub> H <sub>5</sub> -	<i>p</i> -C <sub>6</sub> H <sub>4</sub> NO <sub>2</sub>	202	C <sub>15</sub> H <sub>11</sub> O <sub>2</sub> N <sub>3</sub> S	13.6	14.1
3	C <sub>6</sub> H <sub>5</sub> -	<i>o</i> -C <sub>6</sub> H <sub>4</sub> OCH <sub>3</sub>	195	C <sub>16</sub> H <sub>14</sub> ON <sub>2</sub> S	10.0	9.9
4	C <sub>6</sub> H <sub>5</sub> -	$\beta$ -C <sub>10</sub> H <sub>7</sub>	127	C <sub>19</sub> H <sub>14</sub> N <sub>2</sub> S	9.1	9.2
5	3 : 4 : 5-(CH <sub>3</sub> CO·O) <sub>3</sub> C <sub>6</sub> H <sub>2</sub> -	H	171	C <sub>15</sub> H <sub>15</sub> O <sub>6</sub> N <sub>2</sub> S Br	6.5	6.5

TABLE II

No.	R <sub>1</sub>	R <sub>2</sub>	m.p. °C.	Empirical formula	Nitrogen Percentage	
					Found	Calculated
1	C <sub>6</sub> H <sub>5</sub>		227	C <sub>24</sub> H <sub>17</sub> N <sub>3</sub> S <sub>2</sub>	10.2	10.2
2	$\beta$ -C <sub>10</sub> H <sub>7</sub>		242	C <sub>32</sub> H <sub>21</sub> N <sub>3</sub> S <sub>2</sub>	8.2	8.2

Org. Chem. Laboratories,  
Bangalore.

Indian Institute of Science,  
September 17, 1948.

M. V. BHATT.

B. H. IYER.

P. C. GUHA.

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### A SIMPLE METHOD OF ISOLATING MUSTARD EMBRYOS AND THEIR CULTIVATION

It was demonstrated by Gregory and Purvis, working with rye and Sen and Chakravarti<sup>2</sup> with mustard that the seat of vernalisation changes is the embryo. The endosperm of rye and cotyledons of mustard do not play any part in the process.

This observation makes it clear, that for an understanding of the mechanism of vernalisation it is the embryo that should be subjected to critical study and for any progress the method of its isolation must be very simple and rapid, and that of its cultivation easy. The method described below for mustard satisfies these conditions.

A small sample of sun-dried large sized mustard seed is taken in a pestle and rubbed lightly with a mortar. Due to pressure the cotyledons break and the embryos get separated. With the help of a slanting piece of glass unsplit seeds are separated from the broken ones and again subjected to the process of rubbing. This could be repeated till there are very few seeds left. The preliminary separation of the embryos from the broken cotyledons could be done either by passing them through a sieve of appropriate mesh or by light winnowing. The final selection has got to be done by careful hand-picking and observing each apparently normal embryo under a dissecting microscope for any cleavage. These embryos could then be stored inside a desiccator

for more than a month without losing their viability.

Out of the seeds of the different varieties tried it was found that yellow *sarson*, T. 102, on account of its thinner testa which is more or less loosely attached to the cotyledons, could be handled more easily than the others.

The embryos were first cultivated in a nutrient medium (Purvis modification of white's nutrient solution plus 2% sucrose)<sup>3</sup>, but soon this method was discarded as they were found to grow directly on soil or even on filter paper soaked with tap water. The isolated embryos were sown in pots containing well manured finely sifted garden soil and watered from below by putting the pots in dishes containing water. The growth of the seedlings, however, was slow as compared to those in the nutrient medium. The technique adopted and precautions taken are just similar to the raising of seedlings of small seeded plants like poppy, tobacco, etc.

An attempt to grow isolated embryos of wheat on the other hand, resulted in utter failure. No growth was observed in tap water until and unless the embryos were supplied with a small quantity of sucrose, while the mustard embryos grew well for some days even in glass distilled water.

This observation is interesting as the percentage of sucrose in the wheat (Pb 9-D) embryo-samples worked with was found to vary from 5.6 to 6.1% while it was absent in the embryos of mustard T. 102. Normally it is the mustard embryo that ought to have required sucrose for germination and not wheat. This presence of sucrose is also essential for the maximum vernalisation of rye embryos<sup>3</sup> but it is not so far the embryos of mustard<sup>2</sup> which, would germinate as well as vernalise quite successfully even in glass distilled water.

I have not so far come across a parallel case and would greatly appreciate if some workers could let me know cases of isolated embryos capable of germination and independent establishment in the absence of any nutrients.

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Department of Botany, S. C. CHAKRAVARTI.  
Balwant Rajput College,  
Agra,  
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