The Journal of Heredity 72:227–228. 1981.

Triticum timopheevii × *Secale cereale* crossability

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ABSTRACT: *Crossability of* Triticum timopheevii × Secale cereale *as influenced by environments and E-Amino-n-Caproic acid (EACA) was categorized under visual seed set (as percent of spikelets pollinated), stimulation, endosperm, embryo, and embryo + endosperm formation—all as percent of seed set. Embryo formation was 30.5 percent in the growth chamber environment, compared to 0 percent in the greenhouse. EACA reduced embryo formation but enhanced values in the embryo + endosperm category. The use of EACA in complex intergeneric hybrids among other Triticeae is discussed.*

INTERGENERIC HYBRIDIZATION studies in Graminae, other than Tritico-secale Wittmack, have gained more importance since the mid-1960's. The resulting practicality to agriculture has been limited to *Triticum* species combinations with *Secale cereale*. A certain degree of homology can be envisioned among these genera, since several *Triticum* species cross more readily with *Secale*. It is feasible to manipulate crossability between *Triticum* and *Secale* by 1) a genetic involvement[18], 2) influence of the polyploidy levels of *Triticum*[11], or 3) exogenous treatment of the maternal parent by animal effective immunosuppressants[20].

Hybrid combinations have been produced within the Triticeae among genera more distantly related than *Triticum* and *Secale*. The frequency of embryo recovery has remained fairly low, however. Intergeneric crossability barriers in such combinations have been circumvented by exploiting genetic variability[4], pre- and/or post-pollination treatment of the maternal plants with growth regulators[5], or by utilizing environmental variability[21] and incorporation of immunosuppresant treatment of the maternal parent[13]. Moss[20] reported significant ovary stimulation/development as an index correlated with fertilization when *T. timopheevii* was pollinated by *Secale*.

The readily visible fertilization marker in *T. timopheevii* × *S. cereale* formed the germ plasm basis in this investigation for determining the effects of environmental variability, and E-amino-n-caproic acid (EACA) on manipulating seed set frequency, embryo and endosperm development.

Materials and Methods

Triticum timopheevii (2n = 4x = 28, AAGG) and *Secale cereale* 'Prolific' (2n = 14, RR) plants were grown in pots filled with soil, where they were maintained in the greenhouse and growth chamber environment. The greenhouse plants received 10 hours of natural daylight, and day/night temperatures of 21.1°C and 15.6°C. The growth chamber conditions were a 16-hour day at 26.6°C and 8-hour night at 15.5°C, 42 percent relative humidity, and 32,000 lux light intensity.

The *T. timopheevii* plants were emasculated in the greenhouse and hand-pollinated with *S. cereale* pollen after 3 to 4 days—depending upon stigma receptivity. After 8 hours of pollination with *S. cereale*, *T. timopheevii* florets were treated with EACA once each day for four days.

Observations on *T. timopheevii* × *S. cereale* crossability recorded 20 days after pollination, were categorized as: 1) seed set (percent of spikelets pollinated); 2) stimulation percent; 3) endosperm percent, 4) endosperm + embryo percent, with 2) to 4) expressed as percent of seed set.

Results and Discussion

There are various findings concerning the crossability of wheat with rye and its inheritance[8–10,18,19,23]. Two genes reportedly control crossability frequencies for *T. aestivum* × *S. cereale*. The homozygous recessive, heterozygous, and homozygous dominant associations of the two genes cause crossability values to be high, medium, or low. *T. turgidum* cultivars follow a variable crossability range with rye, but generally give better results than does *T. aestivum*[3]. However, the hybrid embryos (2n = 3x = 21, ABR) germinate poorly[7,10].

T. timopheevii (2n = 4x = 28, AAGG) falls in a low-to-medium crossability class, depending on the rye pollen source. Irrespective of this pollen source, the post-pollination development of the maternal tissue in *T. timopheevii* is extremely pronounced. This has been correlated with fertilization[11]. Visual observation, however, does not provide conclusive evidence of the true fertilization process. It has been referred to by this author as "stimulation", separating it from the "seed set" terminology used by Kruse[6]. The crossability data in Table I elaborate this "seed set" and "stimulation" difference further.

If the greenhouse data were true fertilization, and if seed set were as used by Kruse[6], then a 0 percent embryo recovery should not be expected from the 43.7 percent seed set. This visually observed seed set is instead comprised of a 84.8 percent stimulatory response, with the remaining 15.3 percent due to the presence of endosperm tissue. The total number of spikelets pollinated and the characteristics observed are illustrated in Figure 1.

The results of the growth chamber study

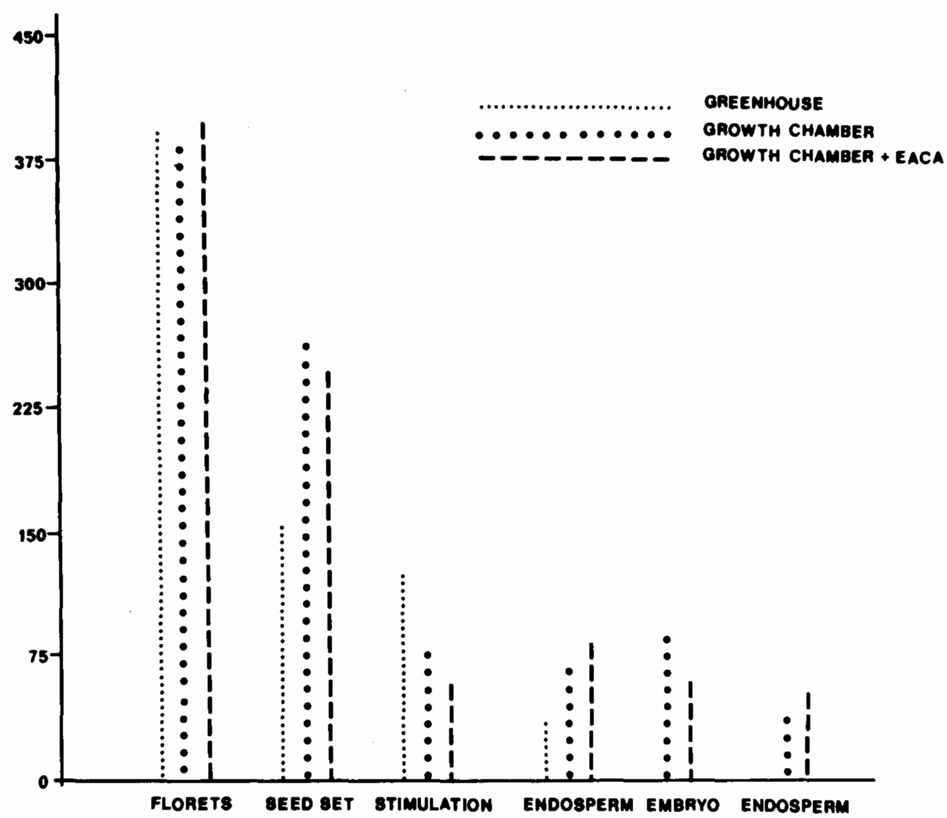

FIGURE 1 Number of *Triticum timopheevii* florets pollinated by *Secale cereale* with crossability responses observed under two environments and EACA influence.

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Table I. Mean *Triticum timopheevii* × *Secale cereale* crossability characteristics as influenced by environment and EACA

Characteristics observed	Growing conditions: greenhouse	growth chamber	growth chamber + EACA
Seed set at % of spikelets pollinated	43.7	70.2	61.9
Stimulation as % of seed set	84.8	27.1	14.5
Endosperm as % of seed set	15.3	19.5	27.0
Embryos as % of seed set	0	30.5	18.9
Embryos + endosperm as % of seed set	0	11.4	18.0

further substantiate this seed set and stimulation trend. A noticeable change in the growth chamber data was a reduction in stimulation associated with an increase of embryo recovery. The optimum environment, with humidity as a major factor, was presumably the major variable accounting for these data shifts.

Kruse's[5] intergeneric crossing technique also demonstrates that microenvironment—particularly humidity—augments hybridization success within the Gramineae. In addition, photoperiodic influences on stigma receptivity may also have aided in eliminating variation, as earlier demonstrated[2,21] for pre- and post-fertilization investigations among some Triticeae.

Taira and Larter[21] indicated that EACA, an immunosuppressant, supported embryo development in *T. turgidum* × *S. cereale* crosses. In the growth chamber phase of the *T. timopheevii* × *S. cereale* crosses, EACA reduced embryo recovery from 30.5 percent to 18.9 percent (Table I); but it increased the embryo + endosperm category from 11.4 percent to 18 percent. This subtle contribution may have a major influence on embryo development and its post-pollination recovery schedule. The longer the embryo is nourished in the maternal tissue with endosperm support and attains size, the easier are the processes related to handling of the embryo during extraction, differentiation of the embryo in embryo culture, and formulation of the embryo culture media. The 6.6 percent increase is attributed primarily to the optimum growth chamber environment.

Although the functional analogy of EACA effects can be based on the results of hybrid formation in dicotyledonous[1] and monocotyledonous systems, positive effects within each system may not be the norm. EACA aided pod development in the mung bean × rice bean crosses[1] and influenced embryo development in *Triticum* × *Secale* crosses[20] in monocotyledonous Triticeae, but in crosses involving some other Triticeae, EACA effects were nonsignificant. The combinations between some species of *Elymus*, *Hordeum*, and *Triticum* do not demonstrate enhanced embryo recovery from EACA treatment. Among these combinations gibberellic acid (GA), a growth hormone, yielded a positive response. Sterile F_1 hybrids, backcrossed to a desired parent and treated with GA[12,13], yielded backcross seed in combinations of: (*H. vulgare-T, aestivum*) × *T. aestivum*[14,15], (*H. vulgare-T. turgidum*) × *T. turgidum*[12,16], and (*T. aestivum-E. giganteus*) × *T. aestivum*[17]. EACA treatment gave no backcross seed[14,15]. The above findings lead this author to conclude that crossability barriers within the same family offer a differential response to exogenous manipulation and are assisted by environment. Potential use of EACA appears to be restricted to production of F_1 hybrids between *Triticum* and *Secale*, especially for cultivar combinations where the genetic crossability system may express a barrier.

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