Survival, growth retardation and malformations after treatment with $CdCl_2$ and/or radiation on day 8 of gestation. Examination on day 13. Only statistically significant differences are entered

Treatment $CdCl_2$ (mg/kg)	Radiation (Gy)	No. of litters	Live fetuses No. (%)[a]	Growth retardation No. (%)[b]	Malformations Total No. (%)[b]	Exencephaly	Eye	Others
–	–	33	413 (90.0)	38 (9.2)	45 (10.9)	0.5%	6.3%	4.1%
2.0	–	14	190 (92.7)	18 (9.5)	104 (54.7)[c]	35.8%[c]	18.4%[c]	0.5%
–	0.5	25	287 (90.5)	21 (7.3)	67 (19.9)[c]	1.5%	15.0%[c]	3.8%
2.0[e]	0.5	18	238 (90.5)	40 (16.8)	54 (22.7)[d]	8.0%[d]	12.6%	2.1%
2.0[f]	0.5	16	180 (90.0)	11 (6.1)	86 (48.0)	22.2%	23.5%	2.2%
–	1.0	24	266 (90.8)	29 (10.9)	110 (41.4)[c]	12.0%[c]	25.9%[c]	3.4%
2.0[e]	1.0	17	219 (95.2)	33 (10.5)	98 (44.7)	16.4%	27.3%	1.0%
2.0[g]	1.0	14	134 (81.2)	18 (13.4)	92 (68.7)	18.7%	50.0%	–

[a]Percentage of implantations; [b]percentage of live fetuses; [c]$p < 0.02$ compared to NaCl-control; [d]$p < 0.02$ compared to $CdCl_2$ alone; [e]30 min before irradiation; [f]60 min before radiation; [g]immediately after irradiation.

treated group). The proportion of microphthalmia and anophthalmia also reached a subadditive level except when $CdCl_2$ was administered immediately after X-irradiation. In this case a synergistic relation exists between the two agents involved. Among the other malformations mainly tail defects, one spina bifida (Cd plus 1.0 Gy) and one duplicitas posterior (Cd plus 0.5 Gy) were observed. It may be noted that combinations of cadmium and radiation showed no significant interaction concerning lethal or growth retarding effects.

Discussion. These results confirm the teratogenic activity of cadmium in animal experiments exerting a significant increase in the rate of exencephaly and eye anomalies. Exposure of mouse embryos to a single dose of 0.5 or 1.0 Gy X-rays resulted mainly in microphthalmia, as the only type of eye malformation. Coadministration of the heavy metal with X-rays on day 8 of gestation in mice resulted in a interesting antagonism of teratogenesis. It is evident that the frequency of exencephaly, a severe defect of the central nervous system, is smaller in all combined treated groups than in the corresponding Cd-group. The antagonistic effect of radiation and cadmium was generally most pronounced when the time interval between the application of the two agents was 30 min. This finding suggests that the specificity of the teratogenic interaction is time-dependent, and may explain the varying degrees of antagonism in brain and eye damage. Concerning the induction of eye malformations the antagonistic action of cadmium with irradiation was less pronounced compared to that of exencephaly. In one case, where $CdCl_2$ was given immediately after 1.0 Gy, even a supra-additive response with respect to the development of eye damage has been found. This synergism may be explained by the absence of an adequate protection mechanism against the disturbance of the eye morphogenesis.

Protection against the teratogenic action of cadmium was also achieved with zinc, selenium, mercury and pretreatment with cadmium[9]. It is postulated that this protective effect may be due to the induction of maternal synthesis of metallothionein[14]. This protein binds cadmium and may prevent the embryotoxic effects during sensitive stages of development. This hypothesis and further possible explanations for the observed antagonism are the subject for our future work.

Acknowledgments. This research was supported by the Swiss National Foundation for Scientific Research, grant Nr. 3.126-0.81.

1 Bernard, A., and Lauwerys, R., Experientia *40* (1984) 143.
2 Hallenbeck, W.H., Experientia *40* (1984) 136.
3 Degraeve, N., Mutat. Res. *86* (1981) 115.
4 Levin, A.A., and Miller, R.K., Teratology *22* (1980) 1.
5 Machemer, L., and Lorke, D., Toxic. appl. Pharmac. *58* (1981) 438.
6 Messerle, K., and Webster, W.S., Teratology *25* (1982) 61.
7 Schowing, J., Acta morph. hungar. *32* (1984) 37.
8 Tassinari, M.S., and Long, S.Y., Teratology *25* (1982) 101.
9 Ferm, V.H., and Hanlon, D.P., in: Reproductive and Developmental Toxicity of Metals, p. 383. Eds T.W. Clarkson, G.F. Nordberg and P.R. Sager. Plenum Press, New York 1983.
10 Ueda, K., J. Radiat. Res. *16* (1975) 90.
11 Müller, W.-U., Streffer, C., and Kaiser, U., Archs Toxic. *51* (1982) 303.
12 Michel, C., and Fritz-Niggli, H., in: Late biological effects of ionizing radiation, vol. 2, p. 397. I.A.E.A., Vienna 1976.
13 Michel, C., Cordt-Riehle, I., Fritz-Niggli, H., and Schäppi, K., Radiat. envir. Biophys. *21* (1983) 155.
14 Layton, W.M. Jr, and Ferm, V.H., Teratology *21* (1980) 357.

0014-4754/86/010080-02$1.50 + 0.20/0

Ontogenetic changes in isozyme patterns during seed germination of self-pollinated *Secale* species[1]

F.J. Vences, F. Vaquero and M. Pérez de la Vega

Departamento de Genética, Facultad de Biología, Universidad de León, E-24071 León (Spain), 8 May 1985

Summary. Six isozymatic systems have been studied comparatively during the first week of germination of seeds of self-pollinated *Secale* species (*S. silvestre* Host. and *S. vavilovii* Gross.). Isozymatic systems do not change at all, or reach their definitive adult plant pattern early during germination.
Key words. Secale silvestre; *Secale vavilovii*; isozyme changes; seed; germination.

The expression and/or the activity level of particular isozymes, and sometimes all the isozymes of an isozymatic system, are often related to a tissue, an organ or a developmental stage[2,3]. Differences in isozymatic patterns among different tissues and organs, or during development, have been attributed to one or more of the following causes: 1) changes in the level of isozyme expression; 2) genetic transcription of different isozyme loci; and, 3) post-translational isozyme modifications changing the electrophoretic mobility. Many examples of organ specificity, or changes during development, or isozyme patterns have been reported in higher plants, including cereals. For the latter there are several reports, for example of organ specificity in *Triticum*[4] and *Secale*[5,6], and of changes during development[7-9]. On the other hand, the expression of different isozyme loci at different developmental stages or in different organs, and changes in electrophoretic mobility which facilitate the isozyme resolution

in gels, are of further interest in genetic studies other than developmental genetics, since they can supply additional genetic markers for many such studies (e.g. linkage and gene mapping studies, phylogenetic relationships).

The use of a genetically uniform material is an important factor in studies on the isozymatic variability during development because heterogeneity among samples or individuals can be eliminated as a possible cause of the variation observed. Four species are generally accepted in the genus *Secale*[10]. *S. cereale* and *S. montanum* are open-pollinated species and therefore genetically variable for most of the isozymatic systems assayed[11], whereas *S. silvestre* and *S. vavilovii* are self-pollinated species and highly homogeneous within each population. Thus, the variation among plants in the two former species makes the study of ontogenic changes of isozymes systems difficult; however, the changes of some isozymatic systems during development in rye (*S. cereale*) inbred line seedlings have been studied[6].

The purpose of this work is to study comparatively the ontogenic changes of six isozymatic systems during the germination of both *S. silvestre* and *S. vavilovii* seeds. The patterns found during the first week of germination are compared with patterns described in dormant seed and adult plants.

Materials and methods. Seeds of *Secale silvestre* Host. and *S. vavilovii* Gross. were germinated on moist filter paper under a 16 h light photoperiod at 20 °C. Samples (four seeds every time) were taken from 0 to 106 h of germination. Four parts of each seed/seedling were separately analyzed; namely, embryo and scutellum (E+S), endosperm (Ed), coleoptile and mesocotile (CM) and roots (R). Single seed extracts were obtained after crushing the tissues in a small vial (5 µl per E+S and 20 µl per Ed of sodium acetate 0.1 M, pH 7.2, were added during 1 h at 2 °C). Paper wicks were soaked with the crude extract and inserted in 12% w/v horizontal starch gels. The isozyme systems assayed were: glutamic-oxaloacetic transaminase (GOT, EC 2.6.1.1); phosphoglucose mutase (PGM, EC 2.7.5.1), phosphoglucose isomerase (PGI, EC 5.3.1.9), malic dehydrogenase (MDH, EC 1.1.1.37), acid phosphatase (ACP, 3.1.3.–), and cathodal peroxidase (CPX, EC 1.11.1.7). Electrophoretic and staining procedures have been previously described[12]. Ungerminated (0 h) seed parts (E+S and Ed) were always run as electrophoretic controls in gels. Isozyme and genetic nomenclature follow, with minor modifications, the ones described by Pérez de la Vega and Allard[12]. When they were known, isozymes have been referred to genetic loci; when not, they have been referred to electrophoretic mobility zones.

Results and discussion. Although four species are generally accepted in the genus *Secale*[10,11]; this study has been carried out only in self-pollinated species; therefore samples can be considered to be as uniform. Before the study of germination 100 seedlings (15 days old) of each species sample were electrophoretically analyzed and no intraspecific variation was found. In this way we could reject the influence of genetic heterogeneity on the changes occurring during germination. Unfortunately we had no inbred lines of *S. cereale* and *S. montanum* to enable us to study these species.

The isozyme patterns of root (R) and coleoptile-mesocotile (CM) in each species was the same at each time of sampling. The pattern changes were similar in both species, although it must be taken into account that the emergence of roots and coleoptile are more precocious in *S. vavilovii* than in *S. silvestre*: for instance, after 24 h of germination it is possible to cut off the root meristem in *S. vavilovii*, but only after 44 h is it possible in *S. silvestre*.

The isozyme systems assayed have been classified into three groups according to the kind of modifications they show during germination.

The first group includes the systems which do not change during germination (fig. 1) and show the same pattern in all four parts analyzed; furthermore, these patterns are identical with the patterns of adult plant leaves. This group comprises: 1) GOT: Both species showed the same pattern as was most frequently shown by *S. cereale*[12]. This pattern has three isozymes designated 6.3 (*Got 1*), 5.7 (*Got 2*) and 3.8 (*Got 3-11*) and 4.4 (*Mdh 3-11*). Isozymes 3.8 and 6.3 decreased in intensity from the dormant embryo to R and CM. The endosperm (Ed) pattern seems not to be altered during the time of the experiment. 2) PGM: One interspecific difference was observed; *S. vavilovii* showed isozyme 6.0 (*Pgm-22*) while *S. silvestre* has isozyme 5.8 (*Pgm-11*). 3) PGI: Both species showed the wide band of the *Pgi 1* locus, while *S. vavilovii* and *S. silvestre* had in the *Pgi 2* locus the genotypes *11* and *22*, respectively. 4) MDH, which does not present interspecific differences in its three isozyme loci, 6.4 (*Mdh 1-11*), 5.1 (*Mdh 2-11*). Salinas[13] also found no change in these isozymatic systems during the germination of rye inbred lines. Therefore, these systems behaved as constitutives since they do not change either during germination or among the organs studied. Certainly the enzymes of these enzymatic systems play important roles in the central metabolism of plants. Another indication of their importance may be deduced from the pattern of similarity existing among all *Secale* species, the only difference being the *Pgm* and *Pgi 2* alleles they are fixed for.

The second group includes only phosphatases, which change during germination except for Ed (fig. 2A). *S. vavilovii* Ed always showed three isozymes (4.6 = *Acp 2-22*, and 1.6 and 1.1 related to zone 4). However, *S. silvestre* Ed of dry seed (0 h) has four isozymes, but two additional isozymes appear during the first two hours of germination (5.0 and 5.6, zone 2). The rapid appearance of these two isozymes could be due to a mobilization of stored isozymes previously synthesized during seed formation; therefore we consider this system as invariable. Anyway, isozymes 5.6 and 5.0 must be controlled by the *Acp 2* locus, which controls the same isozymes in the adult plant[11,12]. A similar explanation has been given for changes in wheat peroxidases[14]; the authors suggested that the new isozymes appearing during germination could be due to an increase in activity of former isozymes rather than to the formation of new isozymes. The *S. vavilovii* E+S pattern shows six isozymes (5.4, 5.0 and 4.6 controlled by *Acp 2-22*, and 1.6, 1.1 and 0.6 of zone 4) and two more (zone 1) appear when R and CM emerge (about 24 h), while *S. silvestre* E+S shows only two isozymes increasing to four, also when R and CM emerge. The expression of phosphatase isozymes of zones 3 and 1 of R, CM and adult plant is species specific: *S. silvestre* is homozygous for the *Acp 3-1* allele (isozyme 3.0) while *S. vavilovii* must be homozygous for the null allele *Acp 3-3* (no band)[12]; *Acp 1* phosphatases (7.2, 6.8) are only shown in *S. vavilovii* although they have also been described in *S. cereale*[12].

The last group only includes cathodal peroxidases (*Cpx*), the patterns of which also change in endosperm (fig. 2, B and C).

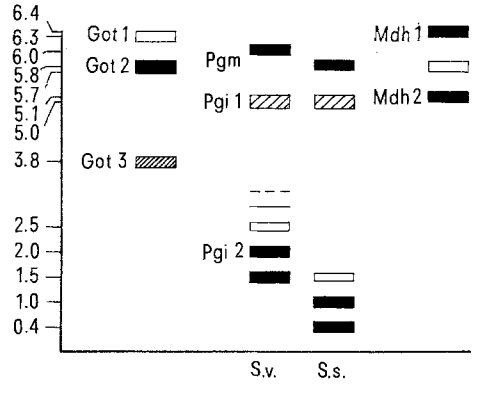

Figure 1. Patterns of invariable isozymatic systems during seed germination of *S. silvestre* (S.s.) and *S. vavilovii* (S.v.). Numbers on the left column denoted each isozyme.

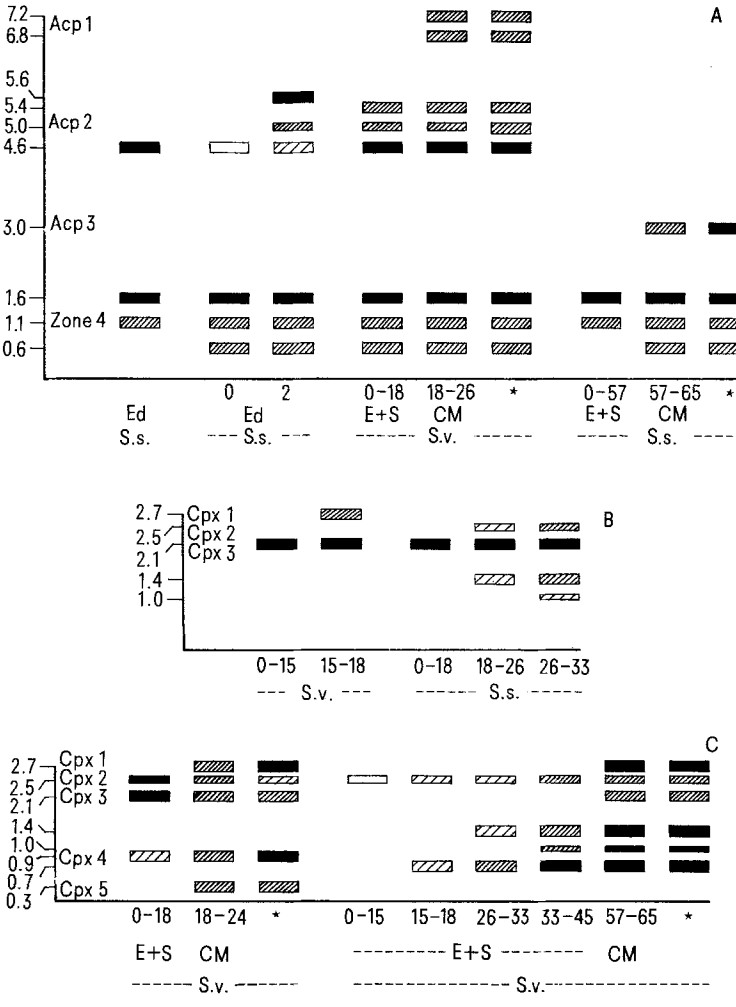

Figure 2. Patterns of variable isozymatic systems. *A* phosphatases; *B* endosperm cathodal peroxidases; *C* embryo (E+S), coleoptile (CM) and adult plant (*) cathodal peroxidases. Numbers under each pattern indicate hours from the start of germination.

Dry seed Ed of both species show the same isozyme (2.1, *Cpx3* locus), but *S. vavilovii* shows only one new isozyme (2.7, *Cpx 1*) after 15–18 h of germination, while *S. silvestre* shows three additional isozymes (2.5, *Cpx 2*, and 1.4 after 18–26 h, and 1.0 after 26–33 h) (fig. 2B). The dry E+S of *S. vavilovii* shows three isozymes (2.5, 2.1 and 0.9 related respectively to loci *Cpx 2, 3* and *4*), and when R and CM emerge two more isozymes are expressed (2.7, *Cpx 1* and 0.3, *Cpx 5*). The dry E+S pattern of *S. silvestre* has only a single isozyme, and three more appear during early germination (0.7, 1.4 and 1.0 after respectively 15–18, 26–33 and 33–45 h; these isozymes are specific for this species); when R and CM are distinguishable after 57–65 h two more isozymes appear (2.7, 2.1) (fig. 2C). It is worth pointing out that there is no clear correspondence between the isozyme bands of E+S, R and CM observed in starch gels in this experiment and the isozymes described in *S. cereale* and *S. vavilovii* using polyacrylamide gels made with Tris buffer[5]. A similar increase in isozymes has been reported in inbred lines of rye[13] and in coleoptiles of wheat[15]. The appearance of new peroxidase patterns has been previously related to a differential genetic programming and/or to post-translational modifications of previous isozymes[7,16]. In our materials this latter cause can be rejected since the appearing isozymes are present in seedlings and adult plant and are controlled by different loci.

In conclusion, it can be stated that in the species here studied there is either no change or an increase in the number of isozymes during germination, the definitive adult plant patterns being reached after approximately 3 days of germination. Roots and coleoptile always showed the same isozyme patterns.

1 Acknowledgements. This work was carried out at the Dpto. de Genética, Univ. Complutense, Madrid and supported by grants from the C.A.I.C.Y.T. (1789–82) and the P.F.P.I.

2 Market, C.L., Isozymes: Current Topics in Biological and Medical Research, vol. 7, p. 1. Eds M.C. Rattazzi, J.G. Scandalios and G.S. Whitt. Alan R. Liss Inc., New York 1983.
3 Scandalios, J.G., Physiological Genetics, p. 63. Ed. J.G. Scandalios. Academic Press, New York 1979.
4 Asíns, M.J., Benito, C., and Pérez de la Vega, M., Z. PflZücht. *89* (1982) 121.
5 García, P., Pérez de la Vega, M., and Benito, C., Theor. appl. Genet. *61* (1982) 341.
6 Salinas, J., and Benito, C., Z. PflZücht. *93* (1984) 115.
7 Asíns, M.J., Benito, C., and Pérez de la Vega, M., Can. J. Bot. *61* (1983) 3393.
8 Endo, T., Biochem. Genet. *19* (1981) 373.
9 Lai, Y.K., and Scandalios, J.G., Dev. Genet. *1* (1980) 331.
10 Khush, G.S., Z. PflZücht. *50* (1963) 32.
11 Vences, F.J., Ph.D. Diss., Univ. Complutense, Madrid 1984.
12 Pérez de la Vega, M., and Allard, R.W., Can. J. Genet. Cytol. *26* (1984) 308.
13 Salinas, J., Ph.D. Diss. Univ. Complutense, Madrid 1983.
14 Kruger, J.E., and LaBerge, D.E., Cereal Chem. *51* (1974) 578.
15 Chappet, A., and Dubouchet, J., Physiol. Veg. *13* (1975) 153.
16 Van den Berg, B.M., Wijsman, H.J.W., and Bianchi, F., Theor. appl. Genet. *66* (1983) 173.

0014-4754/86/010081-03$1.50 + 0.20/0