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RAFFINOSE CONTENT AND VIABILITY AND VIGOUR OF WHEAT GRAINS

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RAFFINOSE CONTENT AND VIABILITY AND VIGOUR OF WHEAT GRAINS

by

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Raffinose content and viability and vigour of wheat grains

A three-month pilot project

Interim Report

This interim report presents the major findings generated by the project. A research paper will be prepared for submission to a scientific journal.

ABSTRACT

Twenty one samples of wheat grains graded at different vigour levels by the Official Seed Testing Station, Cambridge (OSTS) were screened for germination, vigour and raffinose and sucrose contents of the embryos prior to germination. The samples exhibited a wide range of vigour as measured by seedling length attained in standard tests, in agreement with the scores obtained from OSTS. Samples having lower vigour ranking also showed lower germination rates and germination capacity ie viability. High-vigour, high-viability samples had a wide range of raffinose and sucrose contents and R:S whereas all the low-viability, low-vigour batches had low values of raffinose and R:S. Analysis of a low viability sample indicated that non-viable embryos had a raffinose content at least 2-3 times less than that of viable embryos. In enforced aging tests, samples having relatively low raffinose contents and R:S lost viability more readily than the high-raffinose, high R:S samples.

These findings strongly suggest that the potential longevity and the vigour of wheat grains is closely associated directly with the raffinose content and R:S values.

INTRODUCTION

Evidence is accumulating that longevity of seeds, either in storage or when subjected experimentally to accelerated aging, is closely dependent on the oligosaccharide content, eg, raffinose (Bernal-Lugo and Leopold 1992;Horbowicz and Obendorf, 1994; Steadman *et al* 1996) The role of raffinose is considered to be to stabilise the sucrose glass which forms as seeds dehydrate during maturation, and also subsequently during the

dry state in storage. Sucrose vitrification is held to be an important element in seed desiccation tolerance and longevity (Horbowicz and Obendorf, 1994). It has been shown that raffinose begins to accumulate in developing wheat embryos as the grains enter the maturation-drying phase (Black *et al*; 1996) in a process which is sensitive to the water status of the grain (Black *et al*; in preparation). hence, it is conceivable that wheat grains acquire different raffinose contents, depending upon their physiological experiences during maturation. If raffinose is indeed an important factor determining viability/vigour such differences might account for the range of viability and vigour values found in different grain samples.
in this pilot project we have investigated the relationship between viability/vigour of wheat and raffinose content of the embryos.

MATERIALS AND METHODS

Samples (21) of wheat grains having different vigour rankings as determined by the Official Seed Testing Station, Cambridge were kindly supplied by Dr R. Coster. Vigour tests were carried out using the rolled towel method at 15°C. Grains were allowed to germinate and grow for 15 days after which longest root length, shoot length and percentage germination were determined. Germination tests were also done with grains on agar in Petri dishes in darkness at 14 C for 15 days after which no further germination occurred.

Embryos (with some covering tissues) were excised from dry seeds for the estimation of raffinose and sucrose contents. After weighing, replicate samples of 10 embryos were homogenized and extracted twice with hot (70 C) 80% ethanol. The two combined extracts were taken to dryness, dissolved in hot (80 C) water and centrifuged to remove debris. Sucrose and raffinose concentrations were measured in extract aliquots using the respective Boehrihger Mannheim kits. Raffinose:sucrose (R:S) mass ratios were calculated.

To determine the soluble carbohydrate content of living and dead embryos, seed samples were set to germinate on agar at 20 C. After 2 days, embryos that had just germinated (radicles 1-2 mm

long) were excised. Non-germinated embryos were also excised: these were classed as "dead", though almost certainly they were "dead-enriched".

Aging of grains was carried out by keeping them in open Petri dishes at 55 C at ambient relative humidity. Samples were taken at intervals for germination testing on agar at 20 C.

RESULTS

Vigour scores and soluble carbohydrate content

Raffinose (R) and sucrose (S) contents showed a wide range of values in relation to vigour score (Fig.1). Embryos of high-vigour grains had R contents of 24.5 - 68.1 μg/mg dw (mean 49.3) and R:S of 0.2 - 1.1 (mean 0.5). Embryos of low-vigour grains (score 4 - 6) contained R in the range 22.1 - 49.8 μg mg dw (mean 31.6) and R:S of 0.19 - 0.15 (mean 0.33). In vigour tests carried out in this laboratory, using mean seedling length as the index, the low-vigour seedlings were produced by embryos with a mean R:S of 0.34 while high-vigour seedlings were from grains whose embryos had a mean R:S of 0.7 (Fig.2).

Germination capacity and soluble carbohydrate content

The low vigour-score grains showed germination values of approx. 40 - 70% while the high vigour-score samples had values of approx. 92 - 100%. The low-germinating group (ie. containing 30 - 60% non-viable seeds) had R contents of 20 -35 μg/mg dw (mean 27): the high-germinating group had R at 25 - 68 μg/mg dw (mean 46). R:S were 0.19 - 0.4 (mean 0.28) and 0.2 - 1.1 (mean 0.58) respectively (Fig.3). Analysis of variance showed that the differences between the low- and high-viability/vigour samples were significant (raffinose, P=0.95; R:S, P=0.95)

An attempt was made to estimate the soluble carbohydrate of the viable and non-viable embryos in the low-viability grain sample. In all cases embryos of grains that had just germinated had higher R contents than their non-germinated counterparts but sucrose values were too variable for reliability (Table 1).In this experiment the non-germinated embryos would include dead ones and probably some late germinators.

Longevity and soluble carbohydrate content

To investigate the relationship between embryo R, S and longevity, grains were aged at 55 C over 110 days. Three types of grain were chosen: a) Low vigour/viability, low R and R:S. b) High vigour/viability, low R and R:S. c) High vigour/viability, high R and R:S. The aim was to determine if the two high-viability samples which had different R and R:S would have different longevities. Tests revealed no changes in germination capacity until after 60 days of aging but by the end of the aging period (110 days) viability (as measured by germinability) had fallen to approx. 10% in the low-vigour/viability low R:S grains, to approx. 30% in the originally high vigour/viability but low R:S grains and to approx. 45 and 83% in the originally high vigour/viability, high R:S grains (Fig.4). A plot of final germination percentage (ie. viability) against original R:S showed a straight-line relationship (R = 0.89) (Fig.5).

DISCUSSION AND CONCLUSIONS

The wide range of vigour values (as scored by OSTS) was confirmed by our own tests. It is clear that many of the low-vigour batches also had a low germination capacity, ie they had suffered various degrees of viability loss. It may be presumed that many of the still viable grains are likely to produce low-vigour seedlings, partly because their germination rates were low: indeed, there is evidence to support this presumption (data not shown). The low vigour/viability grains had relatively low average R and R:S values. There are indications that those embryos of this batch that had retained viability had higher R than those taken as having died (Table 1). We should emphasize, however, that the dead embryos collected in this experiment may have contained some living individuals which were slow to germinate. Also, it is likely that the living embryos had metabolised some R to support their germination and subsequent elongation growth, so the R content obtained is presumably an under-estimate of the starting level. The real differences in R as between viable and non-viable embryos is therefore likely to

be greater than we were able to show. Unfortunately, because of the great variation in the sucrose contents of the treatments, estimates of R:S are not reliable. There was a wide spread of R and R:S in the high viability/vigour grains, several samples of which were in the same low order as in low-viability batches: the mean R and R:S values were, however, significantly higher than those of the low viability/vigour grains. An aging test carried out on the high viability grains with low R showed that these grains had a reduced longevity. It may be the case, therefore, that all the high viability low R, R:S samples would lose their viability more readily than high R, R:S grains. The time available did not permit us to carry out a more detailed examination of the potential longevity of more samples.

In conclusion, the experimental evidence we have obtained points strongly to an association between R, R:S and wheat grain vigour and viability. There was a wide range of R, R:S values among the samples and it would be important to learn how and when the differences arose. One possibility is that raffinose accumulation differed during grain development and maturation. Another possibility is that R variability developed during grain storage, though this is less likely. The findings indicate the importance of the soluble sugars in wheat grain viability and longevity and offer ways of improving viability and vigour such as by manipulating R biosynthesis during grain development, eg. by genetic transformation technology of the key enzymes.

References

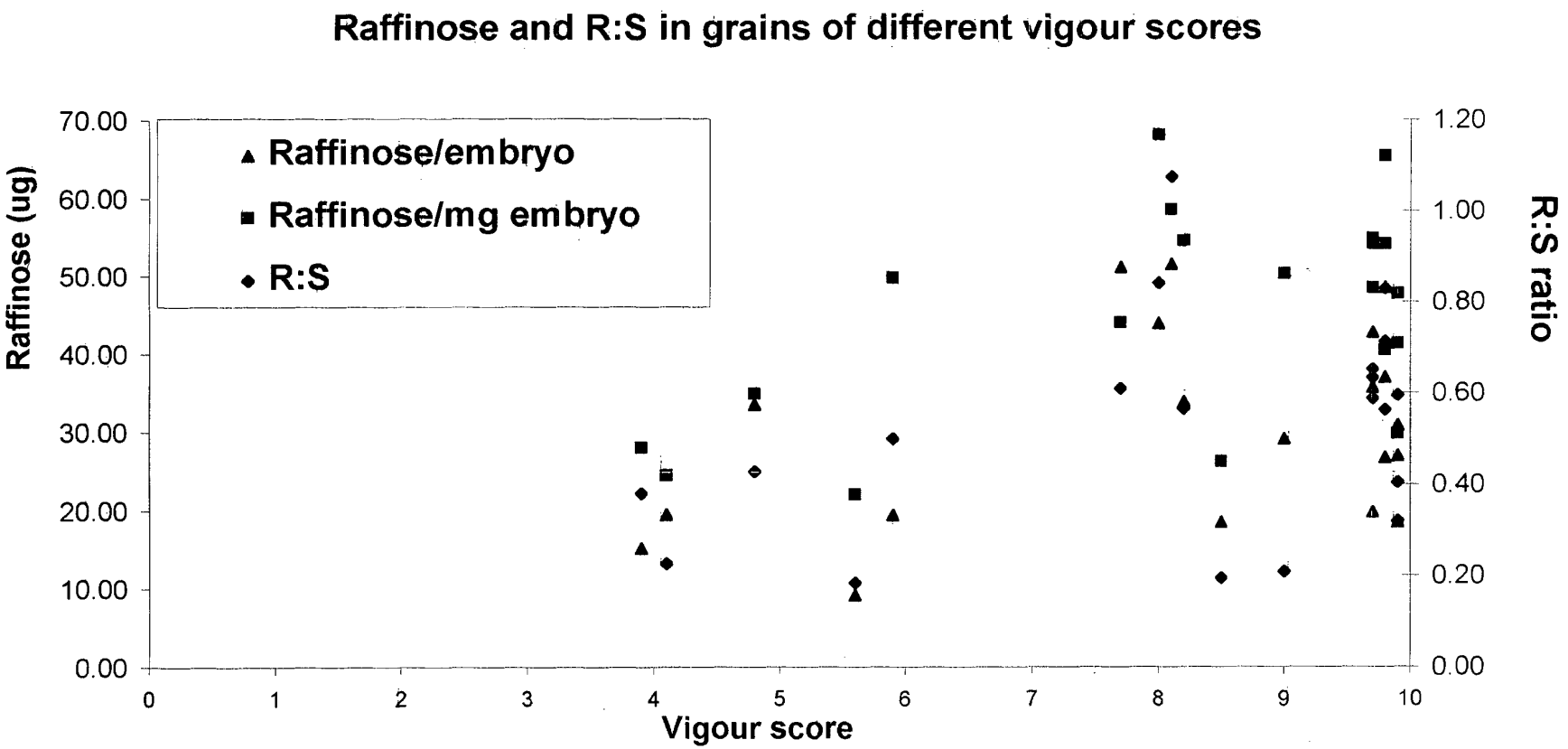
Bernal-Lugo, I. and Leopold, A. C. 1992 Plant Physiology 98, 1207

Black et al. 1996 Journal of Experimental Botany 47, 161

Horbowicz, M. and Obendorf, R. 1994 Seed Science Research 4, 385

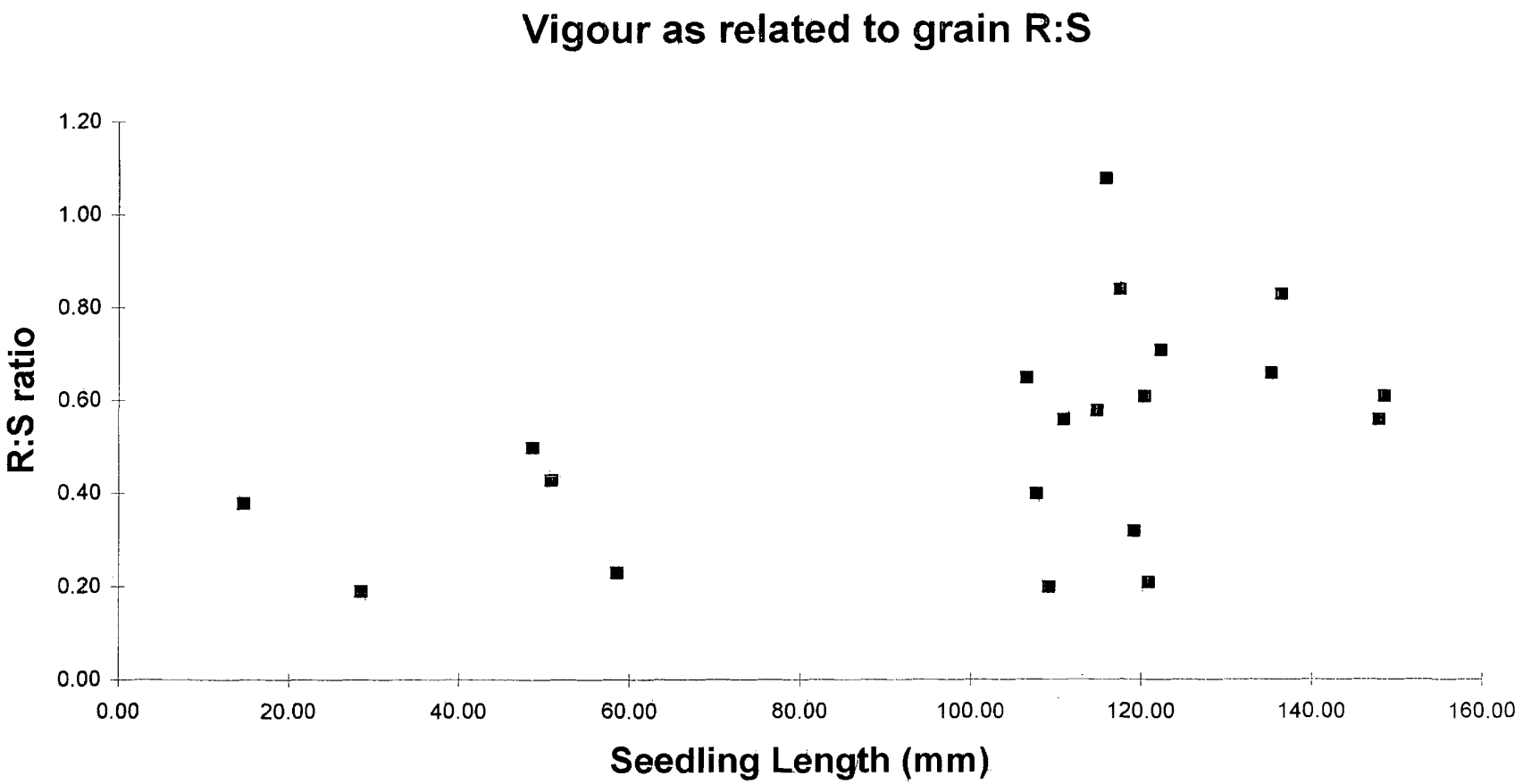
Steadman, K. et al. 1996 Annals of Botany 77, 667

Figure 1.



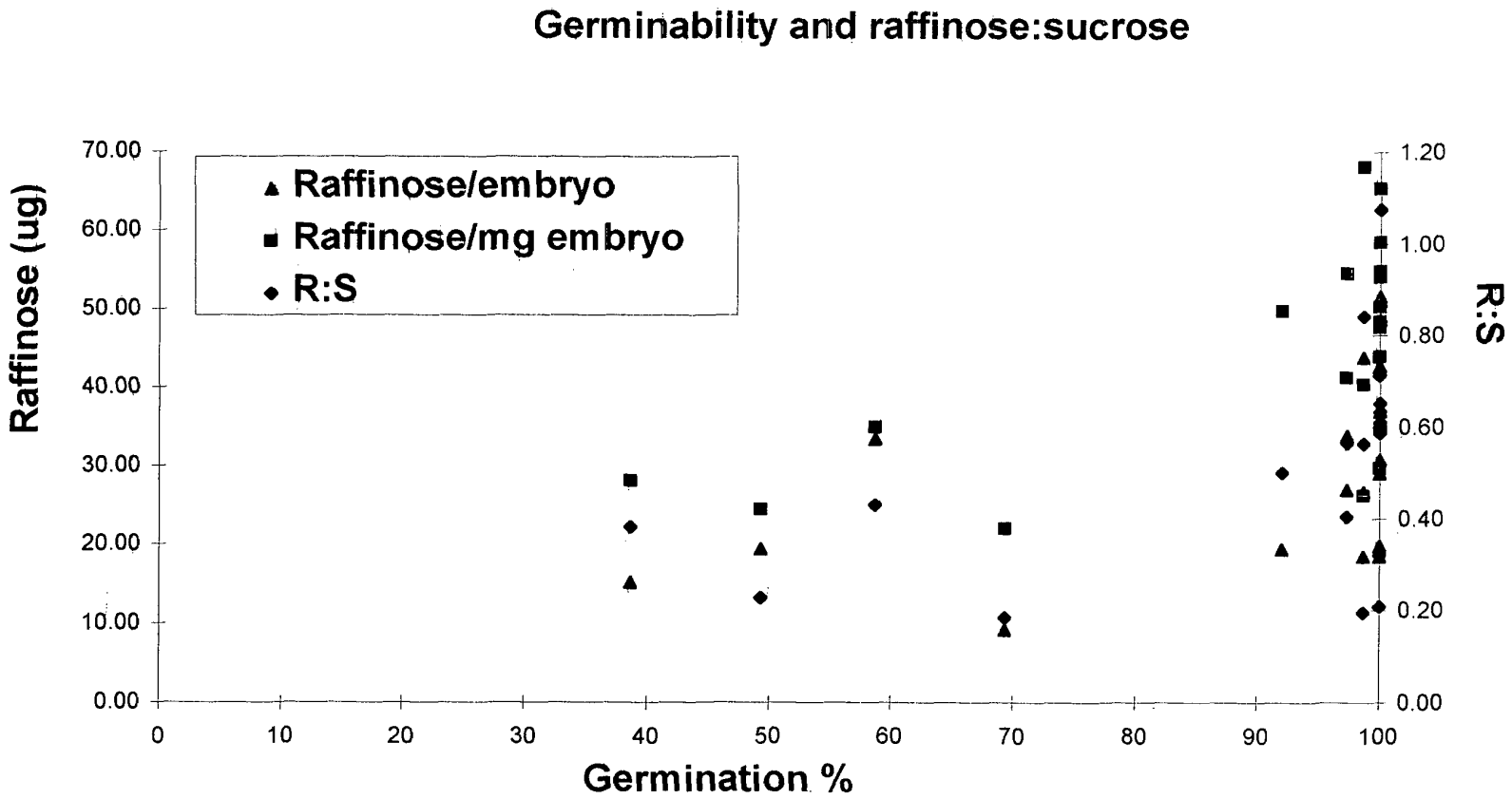
Nineteen samples of differing vigour levels, as scored at the Official Seed Testing Station (high score equals high vigour), were analysed for embryo contents of sucrose and raffinose, from which R:S mass ratios were determined. Values for raffinose are given per embryo and per embryo fresh weight.

Figure 2.



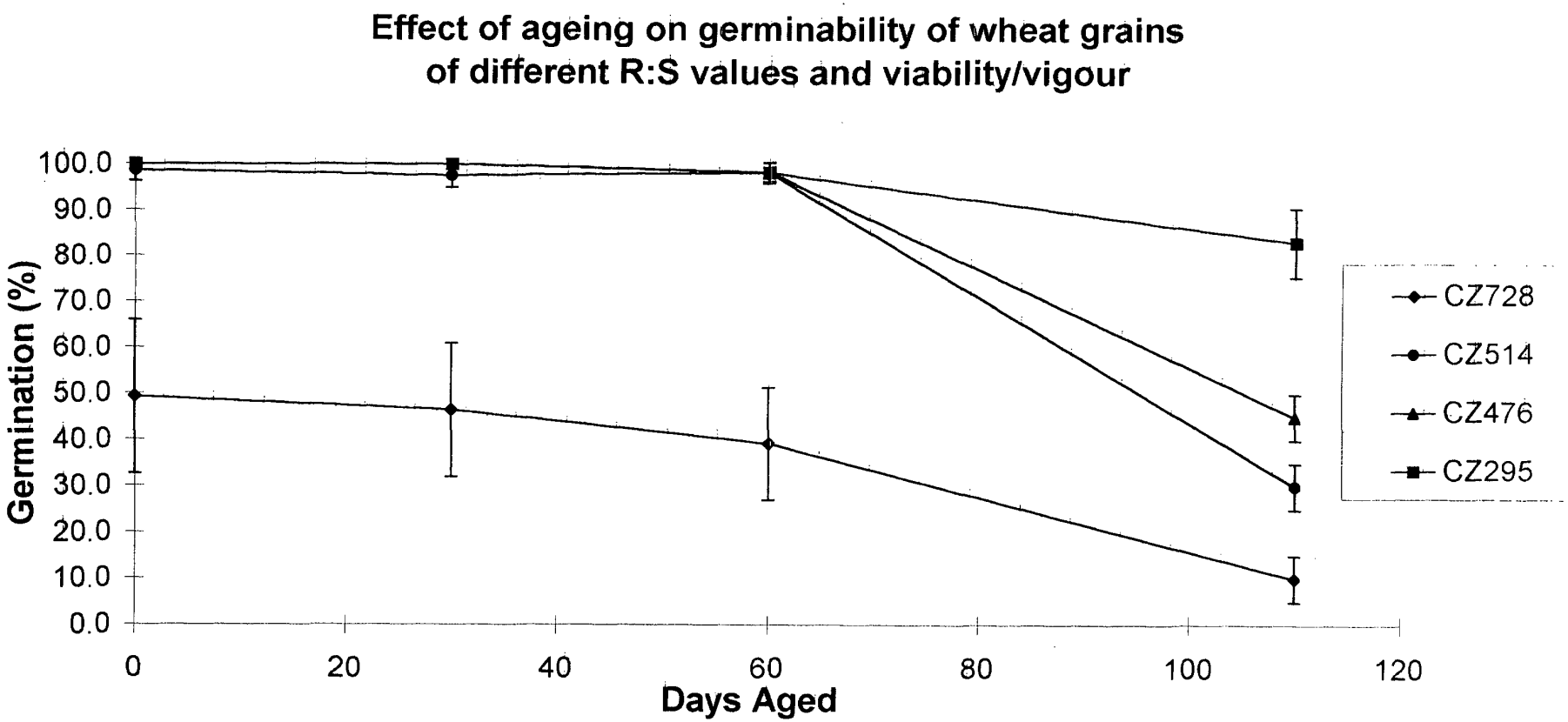
Seeds of different vigour levels were germinated and total seedling lengths achieved after 15 days were measured (tip of longest root to tip of leaf). Lengths are set against raffinose:sucrose (R:S) of the original embryos.

Figure 3.



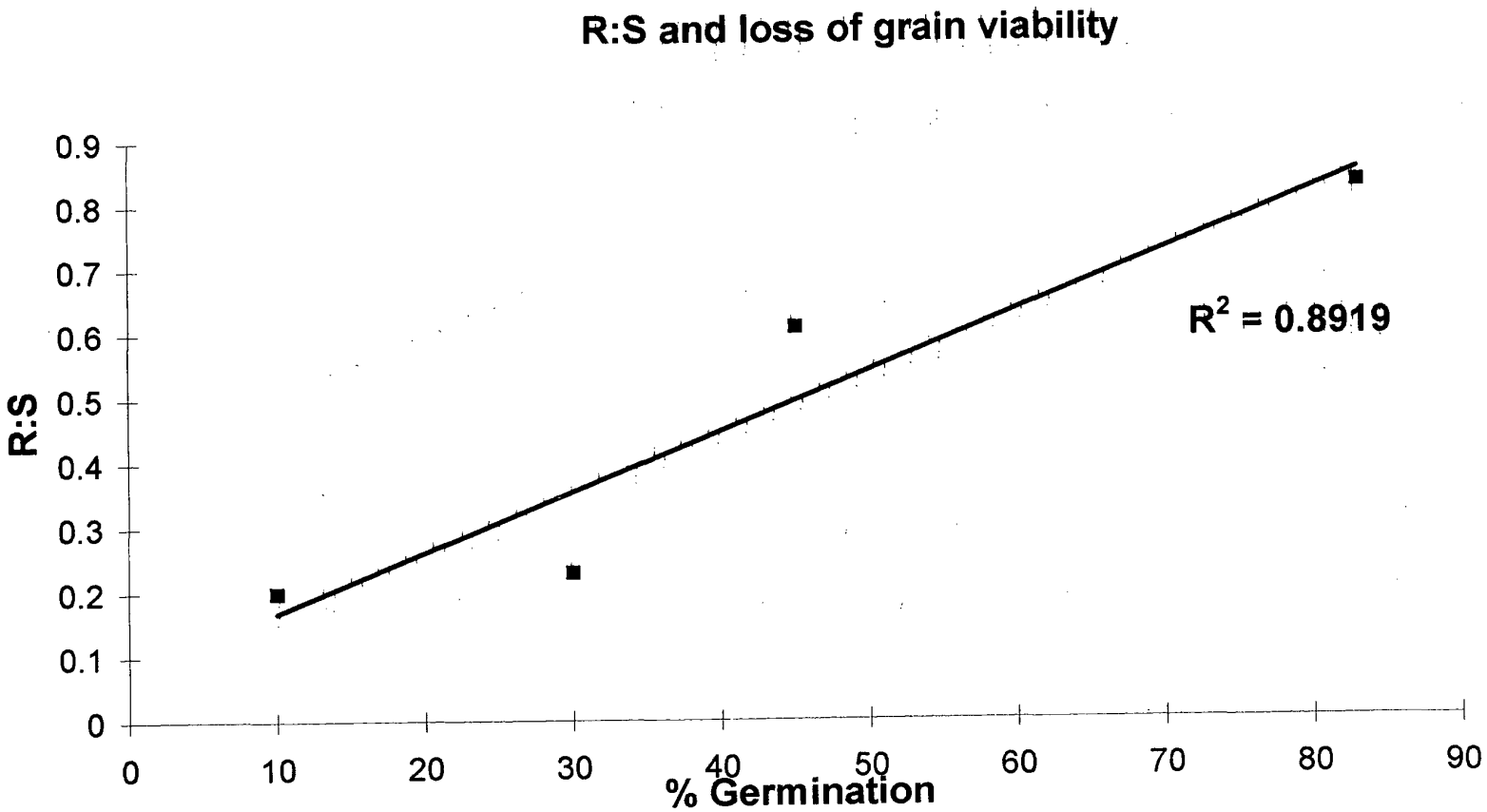
Maximum germination achieved by seeds of different vigour levels was determined. These values are set against raffinose contents (per embryo and per mg fresh weight of embryo) of the equivalent batch of ungerminated seed.

Figure 4.



Grains of four batches differing in mean embryo raffinose:sucrose (R:S) and viability/vigour were aged at 55° C at ambient relative humidity. Samples were taken at intervals to determine viability (%germination). CZ728, R:S 0.23, starting viability 49.5±16.7%; CZ514, R:S 0.2, starting viability 100%; CZ476, R:S 0.61, starting viability 99.5±0.2%; CZ295, R:S 0.83, starting viability 100%.

Figure 5.



Data for raffinose:sucrose (R:S) and viability (final %germination after ageing) taken from figure 4.

Table 1. Raffinose and sucrose levels in living and dead embryos (ug/mg dw)

Sample		Raffinose	Sucrose	Germination
877	living	3.5	114.5	58
	sd	1.0	28.1	
	dead	1.3	80.9	
	sd	0.7	19.3	
728	living	5.3	108.2	49
	sd	2.0	16.5	
	dead	2.5	96.5	
	sd	1.2	14.1	
1001	living	5.0	nd	69
	sd	5.5	nd	
	dead	1.6	nd	
	sd	1.2	nd	

5 repeats mean and standard deviation (sd) (nd=not determined)