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Effects of water deficit during germination of wheat seeds

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Abstract

Germinating seeds of spring wheat (*Triticum aestivum* L.) were tolerant to dehydration up to the 4th day following imbibition and from the 5th day the seedling survival decreased. Dehydration also inhibited the rate of seed dry mass depletion and seedling dry matter accumulation and increased the content of soluble sugars both in grain and seedlings. Glucose supplied either to dry seeds or to 4-d-old seedlings increased survival of dehydrated seedlings. In contrast, exogenously supplied non-readily metabolizable sorbose and mannose suppressed seedling survival.

Additional key words: dehydration tolerance, endosperm reserves, sugars, *Triticum aestivum*.

Introduction

Most of the studies deal with the acquisition of dehydration tolerance in plants at different stages of growth. However, much less is known on loosing this tolerance during germination, which in many species is associated with emergence of the radicle from the seed. As shown earlier by Milthorpe (1950) and later by Blum *et al.* (1980) germinating wheat seeds remain tolerant up to the 3 - 4 d following imbibition, which coincide with rapid growth of coleoptile and appearance of the first leaf.

Some proteins or sugars accumulated during dehydration have been suggested to play a protective role. In many cases, upregulation of late-embryogenesis-abundant (*lea*) genes is associated with dehydration tolerance (Close 1996, Kermode 1997). The content of LEA proteins gradually fall several hours after imbibition (Ingram and Bartels 1996).

Dehydration tolerance is associated with accumulation of certain soluble sugars like raffinose and stachyose which protect the structural integrity of dehydrated membranes and proteins (Hoekstra *et al.* 1997) and also participate in a formation of a glassy state (Leopold *et al.* 1994, Hoekstra *et al.* 1997). Recent evidences indicate that sugars also change the pattern of gene expression (Koch 1996) and regulate cell differentiation, elongation and growth (Wu *et al.* 1993, Weber *et al.* 1996). The genotype and developmental differences in tolerance of spring wheat were found to be related to energy dependent and independent proteolysis (Zagdańska and Wiśniewski 1998). At present, our attention has been focused on the break of tolerance during germination. Therefore, the question has been posed whether the switch from dehydration tolerance to intolerance during early spring wheat seedling growth might be attributed to the global deficiency of available endosperm reserves or to more specific metabolic processes with a special consideration of the modulating effect of exogenous sugars.

Materials and methods

Experiments were carried out on spring wheat (*Triticum aestivum* L., cv. Eta). Grains were surface-sterilised with 1 % NaOCl for 20 min and then rinsed several times with distilled water. After soaking in water overnight at 4 °C, thirty seeds were placed on filter paper in Petri dishes wetted with distilled water. Seeds were allowed to germinate and grow for up to 8 d at 22 °C either in darkness or at the day/night temperature of 22/18 °C with 8-h photoperiod, relative humidity of 60/70 % and photon flux density (PPFD) of 100 μmol m^{-2} s^{-1}. In order to

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Abbreviations: EDTA - ethylenediaminetetraacetic acid disodium salt, HEPES - 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, PPFD - photosynthetic photon flux density, SDS - sodium dodecyl sulphate.

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evoke water deficit in seedlings, water was drained off and the dishes were left open to dehydrate in the growth chamber in dark for 4 d. After this dry treatment, seedlings were rewatered. Three days after rehydration, percent of survived seedlings was calculated as the number of seedlings resuming growth. Seedlings were subjected to dehydration at subsequent days following 24 h imbibition. A similar experiment was performed with the seedlings grown throughout the whole experimental period in the containers with different solutions: A) 2.0 mM KH_2PO_4, 2.5 mM $CaCl_2$, 5.0 mM KCl, 5.0 mM $(NH_4)_2SO_4$, 2.0 mM $MgSO_4$, 0.04 mM ferric citrate and micronutrients, B) 2.5 mM Ca $(NO_3)_2$, 5.0 mM KNO_3, 2.0 mM $MgSO_4$, 1.0 mM KH_2PO_4, 5.0 mM Na_2SO_4, 0.04 mM ferric citrate and micronutrients, C) 5.0 mM KNO_3, 2.0 mM $MgSO_4$, 1.0 mM KH_2PO_4, 2.5 mM $CaCl_2$, 2.5 mM $(NH_4)_2SO_4$, 2.5 mM Na_2SO_4, 0.04 mM ferric citrate and micronutrients and D) different sugars solutions were added as indicated. Germination rate was determined as germination percentage after 4 d at 22 °C in the dark and high humidity. The appearance of a 2 - 3 mm long coleoptile was accepted as the germination criterion.

Water saturation deficit (WSD) in the seedlings was measured according to Stocker (1929) and calculated according to the formula: WSD [%] = (water saturated mass - actual fresh mass) / (water saturated mass - dry mass) × 100, where water saturated mass of the sample was the mass of the sample after submersion in water overnight in the dark and dry mass was determined after drying at 70 °C overnight.

Each day of the experiment about 1 g of tissue of coleoptile, roots or grains were grounded in liquid nitrogen in a cold ceramic mortar. The grain protein fractions were extracted from the defatted powder of the tissue according to Grilli *et al.* (1989). Albumins and globulins were extracted with a 6 cm^3 of 500 mM NaCl, prolamins (gliadins) with a 6 cm^3 of 70 % ethanol containing 0.1 % β-mercaptoethanol and glutenins with a 6 $cm^3$of borate buffer pH 9.0 containing 0.1 % β-mercaptoethanol and 0.05 % SDS. Homogenates were filtered and centrifuged for 10 min at 15 000 g. The protein content in supernatants was measured according to Bradford (1976) using albumin as a standard.

For determination of sugars content about 1 g of fresh tissues of coleoptiles, roots or grains were ground in liquid nitrogen and extracted in 4 cm^3 of 100 mM HEPES, pH 7.0, containing 1 mM EDTA. The homogenate was centrifuged at 15 000 *g* for 10 min. Changes in total soluble sugar content were determined colorimetrically (Dubois *et al.* 1956).

Statistical comparisons were based on the analysis of variance and a significance of differences was computed as the Least Significance Difference (LSD). The mean values obtained were compared by the Tukey's Honestly Significant Difference Test.

Results and discussion

Dehydration tolerance of spring wheat seedlings: Dehydration tolerance was expressed as percentage of seedling survival following severe dehydration of seedlings for 4 d. This treatment led to WSD of about 80 %, irrespective of the seedling age, but seedling survival was 97 - 100 % only up to 4th day of germination and then sharply declined to about 50 % on the 5th day following imbibition. In the following days seedlings survival gradually decreased to about 30 %. Thus, the 5th day may be considered a "break" day in drought tolerance. At that time the coleoptile length was 5.8 and 7.2 cm in the seedlings grown in light and dark, respectively. Within the whole experimental period light had no effect on dehydration tolerance of wheat seedlings. It is evident that there is no direct relationship between water deficit and survival of spring wheat during germination and post-germination growth. Therefore, the observed development of dehydration intolerance seems to be associated more with the stage of seedling growth since water deficit remained unchanged throughout the whole experimental period. During the first two days of germination the intensive cell division and differentiation takes place (Wiedenroth *et al.* 1990) that is accompanied by the high level of dehydration tolerance. On the 3rd day post-imbibition, a phase of intensive growth of coleoptile occurred and appearance of the first leaf on the 5th day coincided with development of seedling susceptibility to dehydration.

Mobilisation of endosperm reserves: The rate of seed dry mass depletion after imbibition was defined as the endosperm utilisation rate (Blum *et al.* 1980). Decrease of grain dry mass was slow up to the 3rd day following imbibition with a mean rate of 0.103 and 0.125 mg h^{-1} for dark and light germinated wheat (Fig. 1*A*). Between the 4th and the 8th day dry mass of dark germinated wheat decreased with a mean rate of 0.156 mg h^{-1} and light enhanced it slightly to 0.170 mg h^{-1}. At the same time, the rate of dry matter accumulation in seedlings was 0.102 and 0.133 mg h^{-1} for dark and light growing seedlings, respectively. These low differences between light and dark grown seedlings may indicate low involvement of photosynthesis in dry matter accumulation in this phase and even photosynthetic gene expression is low in this phase (Loza-Tavera *et al.* 1990, Jang and Sheen 1994).

Dehydration inhibited the endosperm utilisation rate (Fig. 1*A*) and accumulation of seedling dry matter for the seedlings grown either in dark or in light (Fig. 1*B*).

Relative growth rate, estimated on the basis of data given in Fig. 1*B*, attained the highest values of 0.72 and 0.82 g g^{-1} d^{-1} for seedlings on the 4th day of germination.

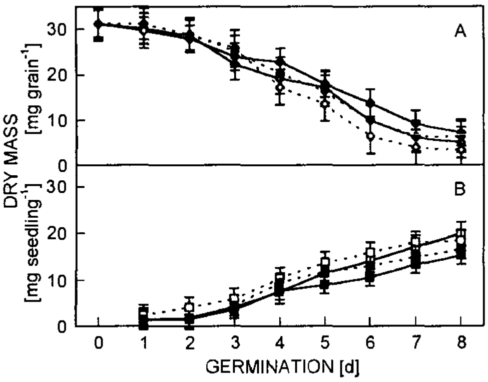

Fig. 1. The time-course of grain reserve mobilisation (*A*) and increase in dry matter of wheat seedlings (*B*) of well watered control (*full line*) and dehydrated at subsequent days following imbibition (*dashed line*). *Open* and *closed symbols* mark light and dark grown seedlings, respectively. Means ± SE, *n* = 9.

The major storage proteins of wheat grain endosperm (Fig. 2) are the alcohol soluble prolamins (52 % of total), followed by alkali soluble glutenins (31 %) and salt-soluble albumins plus globulins (17 %). Participation of particular protein fractions in total storage protein content was still maintained the same as in dry seed. However, after 24 h imbibition the participation of glutenins decreased and albumins and globulins increased in grains of dehydrated seedlings growing in the light. On the 2nd day of germination protein content both in coleoptile (Fig. 3*A*) and roots (Fig. 3*B*) of light growing seedlings was the highest and higher then in dark growing seedlings (Fig. 3*C, D*). Dehydration decreased protein content mostly in coleoptile of both light and dark growing seedlings (Fig. 3*A, C*).

The total soluble sugar content increased practically linearly up to the 4th day representing about 35 % of the total dry mass of grain (Fig. 4*A*). Starting from the 5th day the content of soluble sugars decreased gradually to the level observed in dry seeds. Upon dehydration, the content of soluble sugars transiently increased in the dark germinated seeds whereas in seeds of light grown seedlings it remained on the similar level up to the 3rd day. In the growing seedlings, soluble sugars accumulated almost linearly and on the 8th day their content was higher both in coleoptile and roots of light (Fig. 4*B, C*) and dark (Fig. 4*G, F*) growing seedlings. Dehydration caused an increase in sugar content only in coleoptile of light grown seedlings.

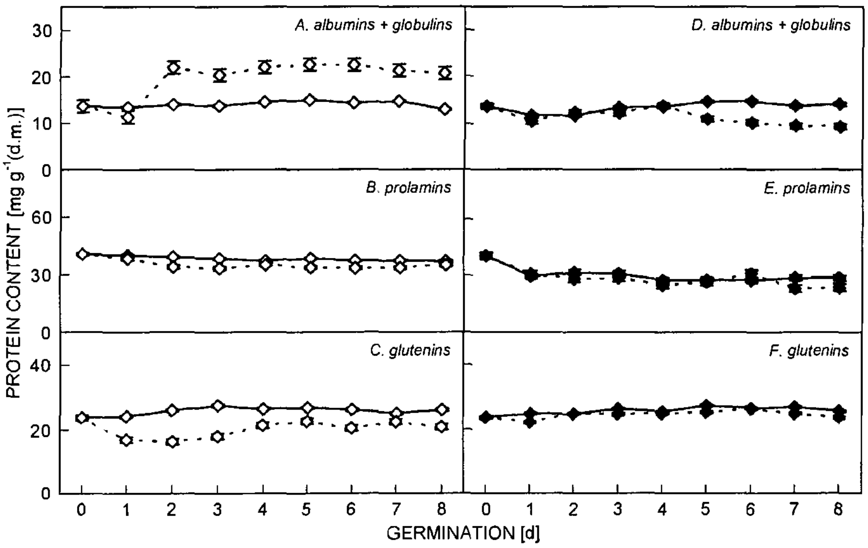

Fig. 2. Changes in the content of protein fractions of wheat grains following germination under optimal conditions (*full line*) and under dehydration (*dashed line*) at light (*A - C*) and dark (*D - E*). Means ± SE, *n* = 9.

The highest relative growth rate was preceded by the intensive mobilisation of seed reserves and followed by the highest content of soluble sugars in grain. These observations point to a rapid turnover of storage compounds in grains and an intensive metabolite export to the growing seedlings. Mobilisation of seed reserves seems to be time-dependent and also dependent on type of storage compounds. Mobilisation of reserve sugars seems to be intensive up to the 4th day of germination. These findings are consistent with the results of previous studies indicating that after an initial day, α-amylase content increased quickly and reach a maximum on the 4th day following imbibition (Corder and Henry 1989).

Dehydration inhibited the rate of reserve mobilisation and delayed accumulation of dry matter with the concomitant increase in sugar content in coleoptile. The main increase in total sugars content in response to dehydration can be attributed to the lowered growth rate. Besides the direct, protective effects, accumulation of sugars contributes to the osmotic potential of tissues that may be of importance for normal seedling development.

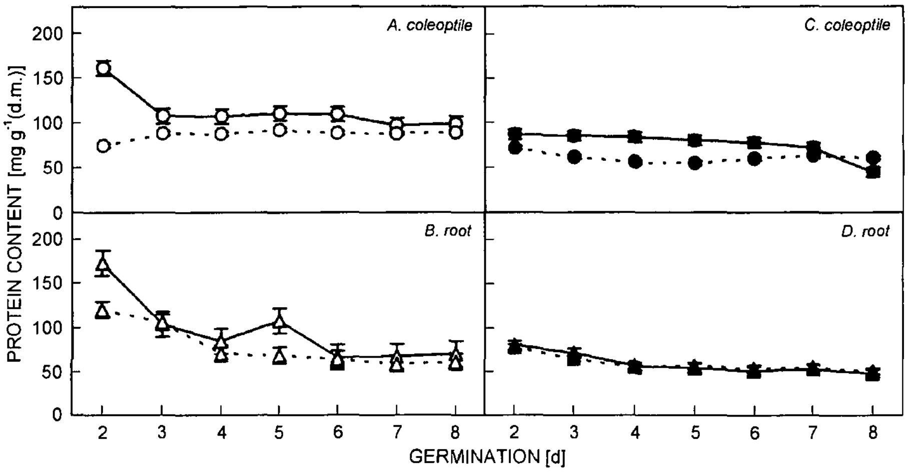

Fig. 3. Changes in the content of soluble protein content in wheat coleoptile and roots of light (*A*, *B*) and dark (*C*, *D*) grown wheat seedlings of well watered control (*full line*) and dehydrated at different days following imbibition (*dashed line*). Means ± SE, $n = 9$.

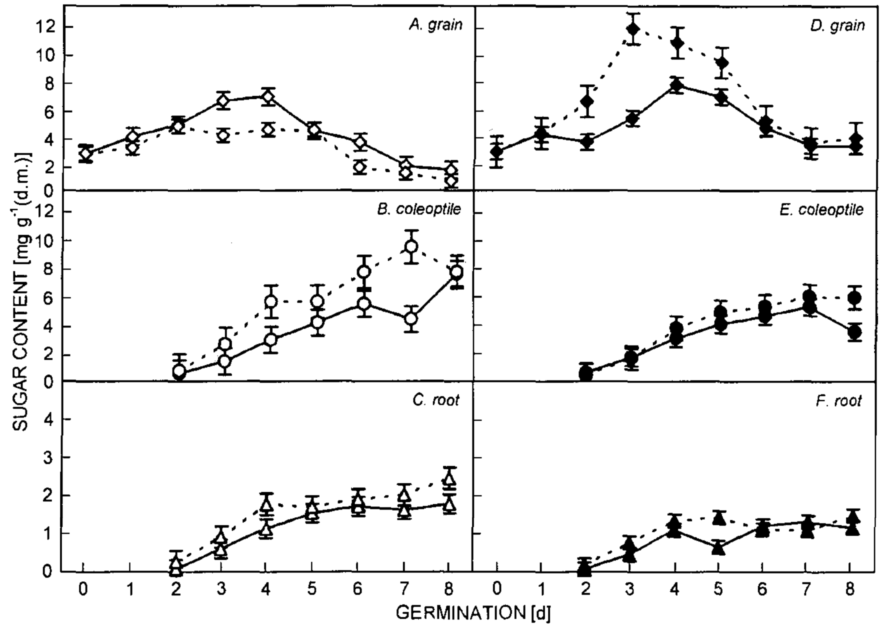

Fig. 4. Changes in the content of soluble sugars in wheat grains, coleoptiles and roots of light (*A* - *C*) and dark (*D* - *F*) grown wheat seedlings of well watered control (*full line*) and dehydrated at subsequent days following imbibition (*dashed line*). Means ± SE, $n = 9$.

Effects of exogenous sugars and nitrogen on seedling survival: To investigate the potential involvement of sugars or nitrogen compounds into dehydration tolerance of seedlings, the effect of different nitrogen sources and glucose on seedling survival following 4 d of dehydration was examined. Nitrogen in the form of NH_4^+ or NO_3^- or both at 200 mM concentration had no effect on seedling survival both in light and in dark up to the 4th day following imbibition. Beginning from the 5th day both nitrate and ammonium nitrogen decreased survival rate equally in dark and light growing seedlings (Table 1).

Exogenously supplied 200 mM glucose to dry seeds increased survival of dehydrated seedlings, the effect being clearly visible for the dark germinating seeds (Table 2). At the same time seedling growth was arrested both in light (Fig. 5) and in dark (not shown). Therefore, we extended our studies to the effect of other hexoses (fructose, glucose, mannose and sorbose) and also sucrose on the survival of seedlings upon dehydration. In preliminary experiments the effect of these sugars at different concentrations on germination rate, *i.e.*, germination percentage after 4 d was examined in order to eliminate the possible inhibitory effect of sugars on post-germinative seedling growth rate (Table 3). In the presence of 5 mM hexoses germination rate did not changed as compared to control seeds germinating in water but sucrose decreased slightly but significantly the percentage of germinating seeds. The 50 mM sorbose and mannose lowered germination to about 40 % while sucrose at the same concentration lowered germination to 69 %. Germination was repressed completely by 100 mM mannose and sorbose and down to 48 % by 100 mM sucrose. Therefore, we investigated the effect of exogenously supplied sugars at 5 mM on survival of seedlings subjected to dehydration following 4 d sugar feeding. Sorbose and mannose depressed by half the survival of dehydrated seedlings, whereas the effect of glucose, fructose and sucrose on seedling survival was negligible. To gain more insight into the possible involvement of sugars in dehydration tolerance we examined the effect of glucose at concentrations ranged from 5 to 200 mM supplied either to dry seeds or to 4-d-old seedlings growing in the light on seedling survival (Table 4). The most effective was 5 mM and 50 mM glucose. Glucose at higher concentrations inhibited the accumulation of dry mass both in coleoptile and roots (Fig. 5) as well as mobilization of reserves.

Table 1. The effect of exogenous nitrogen sources at 200 mM concentrations on survival [%] of light (L) and dark (D) grown wheat seedlings subjected to water deprivation at subsequent days following imbibition. $LSD_{0.05}$ = 11.2 and 11.9 for light and dark grown seedlings, respectively, $n = 9$.

Germination	Control		NO_3^-		NH_4^+		NH_4NO_3	
[d]	L	D	L	D	L	D	L	D
1	100.0	100.0	96.7	100.0	100.0	100.0	100.0	100.0
2	100.0	100.0	96.7	100.0	96.7	100.0	100.0	100.0
3	96.7	100.0	90.0	86.7	86.7	100.0	86.7	93.3
4	90.0	93.3	83.3	86.7	76.7	83.3	80.0	90.0
5	63.3	73.3	50.0	50.0	66.7	70.0	43.3	56.7
6	50.0	63.3	26.7	36.7	46.7	66.7	33.3	40.0
7	33.3	43.3	20.0	26.7	23.3	36.7	13.3	26.7
8	16.7	20.0	6.7	6.7	6.7	16.7	10.0	6.7

Table 2. The effect of exogenously supplied 200 mM glucose on survival of seedlings [%] growing in light (L) or in dark (D) and subjected to water deprivation at subsequent days following imbibition. $LSD_{0.05}$ = 12.7 and 14.6 for light and dark grown seedlings, respectively, $n = 9$.

Germination	Control		Glucose	
[d]	L	D	L	D
1	100.0	100.0	100.0	100.0
2	100.0	100.0	100.0	100.0
3	96.7	100.0	100.0	100.0
4	90.0	93.3	100.0	100.0
5	63.3	73.3	83.3	90.0
6	50.0	63.3	66.7	76.7
7	33.3	43.3	40.0	66.7
8	16.7	20.0	23.3	46.7

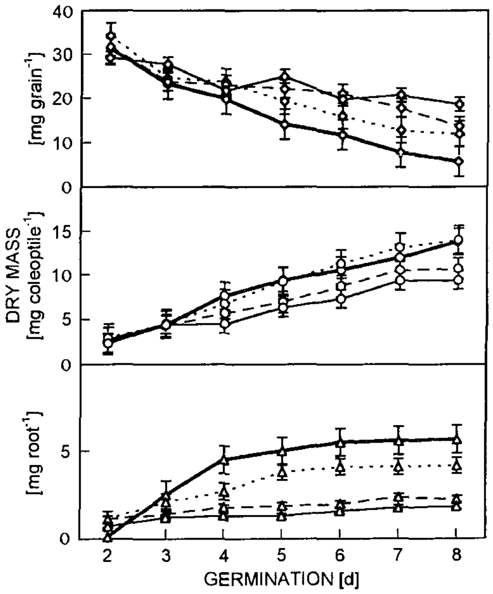

Fig. 5. Effect of different concentrations of glucose on accumulation of dry mass in wheat seedlings. Glucose concentrations used were as follows: control seedlings (*thick full line*), 5 mM (*dotted line*), 50 mM (*dashed line*) and 100 mM (*thin full line*). Means ± SE, $n = 9$.

Increase of tolerance caused by glucose feeding may indicate that the seedlings in the phase of intensive growth require large quantities of ATP. Glucose is an easy metabolizable sugar which may act as a signal for increased respiration, provided cells with carbon and energy for the synthesis of different compounds and ultimately, plant growth (Sturm 1999). Supporting this notion is the observation that 5 mM mannose and

Table 3. The effect of different sugars on germination rate of spring wheat. $LSD_{0.05} = 9.3$, $n = 9$.

	5 mM	50 mM	100 mM	200 mM
Fructose	93.3	89.7	80.3	30.3
Glucose	96.0	91.3	82.7	34.7
Mannose	92.7	37.3	0	0
Sorbose	93.3	42.1	9.2	0
Sucrose	82.7	68.7	48.0	0

sorbose, which are both phosphorylated by hexokinase but only slowly enter metabolism (Walker and Sivak 1986, Smeekens and Rook 1997, Pego *et al.* 2000), decreased seedling survival. Glucose at higher concentrations prevents seedlings against loss of dehydration tolerance by arrested of seedling growth. Glucose concentrations between 5 - 200 mM seems to have no effect on osmotic inhibition of seedling growth because metabolizable sugars inhibit seedling growth at concentrations above 300 mM (Thomas and Rodriguez 1994, Finkelstein and Lynch 2000, Pego *et al.* 2000). It rather indicates that exogenously supplied glucose maintains seedlings at the stage of intensive cell division and differentiation and thus, development of dehydration intolerance is retarded.

Our experiments showed also that there is no relation between germination ability and dehydration tolerance of seedlings. Among sugars investigated at 5 mM concentration only sucrose inhibited the rate of germination but at the same time it increased seedling survival to the level noted for glucose, whereas mannose and sorbose repressed seedling survival but did not arrest germination. One can suppose that hexose phosporylation by hexokinase is not required for germination of wheat as it has been also reported for *Arabidopsis* (Pego *et al.* 1999).

Table 4. The effect of glucose on survival of dehydrated wheat seedlings. Glucose was supplied either to dry seeds (A) or to 4-d-old seedling in light (B). $LSD_{0.05} = 11.8$, $n = 9$.

Germination [d]	Control	5 mM		50 mM		100 mM		200 mM	
		A	B	A	B	A	B	A	B
1	100.0	100.0		100.0		100.0		100.0	
2	100.0	100.0		100.0		100.0		100.0	
3	96.7	100.0		100.0		100.0		100.0	
4	90.0	93.3		93.3		90.0		93.3	
5	63.3	73.3	76.7	70.0	76.3	66.7	70.0	66.7	76.7
6	50.0	56.7	60.0	63.7	66.7	56.7	56.7	53.3	63.3
7	33.3	43.3	53.3	50.0	53.3	43.3	46.7	36.7	43.3
8	16.7	30.0	46.7	36.7	43.3	33.3	36.7	23.3	26.7

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