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Variations in seed quality of primed onion seed lots during storage at -20°C

Ön uygulama yapılmış soğan tohumlarını -20°C'de depolamanın tohum kalitesine etkisi

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ABSTRACT

This work was carried out on twenty onion seed lots to test changes in seed quality during storage at -20°C after priming treatment. Seeds were matric primed at 20°C by mixing seed, vermiculite and water at the ratio of 2:1:3 for 48 hours at 15°C and subsequently dried to 7% seed moisture content. They were then hermetically stored at -20°C over 60 days, and samples were taken after 0 (unstored), 15, 30 and 60 days. Seed germination percentages (20°C, 12 days) and mean germination times were determined. Results indicated that mean germination percentages of the control and primed but not stored seed lots were 80 and 85% respectively. Germination percentages after 15 and 30 days at -20°C remained as high as those of primed and unstored seeds, which were 87%. However, by 60 days after storage, primed seed germination percentages were reduced to 82%, which was non-significant compared to the control. The fastest germination - the lowest mean germination times - were also recorded as 3.64 days after 30 days of storage ($p<0.05$). Results indicated that the benefit obtained from priming may remain in onion seeds for about 30 days after storage at -20°C. Extended storage of up to 60 days can reduce the advantage that is obtained from priming. Storage of primed onion seeds at freezing temperatures can be an alternative method to reduce seed ageing and to maintain high seed quality longer for a certain period.

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ÖZ

Bu araştırmada tohum kalitesinin prime edildikten sonra -20°C'de depolanmış soğan tohumlarındaki değişimi incelenmiştir. Priming uygulaması tohum, vermikulit ve suyun 2:1:3 oranında 48 saat 15°C'de tutularak ardından %7'ye kadar kurutulmasıyla yapılmıştır. Daha sonra her tohum partisinden tohumlar -20°C'de 60 güne kadar hermetik paketlerde depolanmıştır. Bu süreçte 0 (depo öncesi), 15, 30 ve 60 gün sonra örnekler alınmış ve çimlendirme testi (20°C, 12 gün) ve ortalama çimlenme zamanı belirlenmiştir. Kontrol tohumlarında ve prime edilmiş ancak depolanmamış tohum partilerinin ortalama çimlenme oranları %80 ile %85 olmuştur. Prime edilmiş ve 15 ya da 30 gün depolanmış tohumlarda çimlenme oranı %87 olmuştur. Altmış gün depolanmış tohumlarda çimlenme oranı %82'ye düşmüştür ki bu değer kontrol ile anlamlı olarak fark göstermemiştir. Ortalama çimlenme zamanının en düşük olarak saptandığı depolama süresi 3.64 gün ile 30 günlük depolamadır. Sonuçlar prime edilmiş soğan tohumlarının -20°C'de 30 gün kalitelerini koruyarak depolanabildiklerini 60 güne kadar giden depolamaların bu olumlu etkiyi azalttığını göstermiştir. Düşük sıcaklıklarda depolamanın süre dikkate alınarak prime edilmiş soğan tohumları için alternatif bir depolama metodu olduğu belirtilebilir.

1. Introduction

Seed priming is a common post-harvest practice for seed quality enhancement. It induces faster and more uniform seed germination over various stress conditions and in many crop species (McDonald 1999; Demir 2002). During the treatment, seeds are hydrated so that metabolic activities proceed while radicle protrusion is prevented. Then, seeds are dried back to the original seed moisture percentages, i.e. >10% (Demir et al. 2005). In some cases, primed seeds are not sown immediately and need to be stored for a reasonable time when the sowing environment or other conditions are unfavourable. However, primed seeds show a relatively shorter longevity particularly when they are stored in non-optimum conditions. Conclusions on this topic are controversial. Primed leek and onion seeds stored at 10°C retained viability after one year (Dearman et al. 1987). A higher viability was reported in primed tomato seeds over a year at 10°C (Alvarado and Bradford 1988). Sweet pepper seeds maintained viability over three years of storage at 25°C (Thanos et al. 1989). However, some other research does not support these findings. Primed lettuce and leek seeds often have a shorter longevity compared to those of untreated ones (Tarquis and Bradford 1992; Maude et al. 1994; Schwember and Bradford 2005; Ozden et al. 2017). Primed sweet corn seeds had lower germination than unprimed ones after three months of storage (Chiu et al. 2002). Priming delayed tomato seed germination time and reduced germination percentages after six months of storage (Argerich et al. 1989). It appears to be that the effects of priming on longevity may be species specific and depend on the conditions of storage and priming. Various environmental factors affect on the longevity of primed seeds. These can be priming temperature and seed storage temperature (Chiu et al. 2002), relative humidity or seed moisture percentages during storage (Hill et al. 2007), and air composition in the packets (Yeh et al. 2005).

It is known that subzero temperatures of -20°C are used for long term storage for conservation of seeds in gene banks (Roos 1989). It is considered that low temperature keeps the occurrence of seed deterioration during storage at minimum level, and therefore it is used for seed preservation in seed gene banks (Walters et al. 2005). This has been tested previously. Germination percentages of primed sweet corn seeds kept at -80°C over 12 months remained high (Chiu et al. 2002). They showed that anti-oxidative activity seemed to play a preventive role in seed deterioration and loss of viability and vigour. However, the response to storage is species-specific: some species are likely to lose longevity faster than the others (Walters et al. 2005). Onion seed is considered to be sensitive to storability, and pre-sowing treatments are applied for various purposes (Rao et al. 2006). Thus it is likely that primed onion seeds may also be prone to deterioration earlier than untreated ones (Schwember and Bradford 2005). The present study was conducted to test whether the benefit obtained from priming remains during the storage of onion seeds when they were stored at -20°C. Such results may be useful for the seed industry when it is not possible to sow primed seeds straight away.

2. Material and Methods

A sample of 20 onion (*Allium cepa* L.) seed lots belonging to various open-pollinated cultivars were obtained from different seed companies in Turkey. The seeds were stored until use in hermetic, air and water-proof laminated aluminium foil packets at 5°C. The normal seed germination percentages of the seed lots ranged between 65% and 89%. Seed moisture content was determined according to ISTA (2018) and ranged between 7.0% and 7.8%.

The solid matrix priming (SMP) treatment was carried out by mixing seed, vermiculite (No: 5) and water at the ratio of 2:1:3 for 48 hours at 15°C in aluminium foil plastic packets (length 13 cm, width 9 cm) kept in the dark. The weights of seed:vermiculite (No.5):water were 2.5 g, 1.25 g and 3.75 g respectively. After the treatment, the seeds were separated from the vermiculite and then dried at 25°C. Post-priming seed moisture was adjusted to 7±0.5% by weighing seeds.

The treated and untreated (control) seeds were germinated at 20°C between wet paper towels (20 x 20 cm, Filtrak, Germany) for 12 days (ISTA 2018) in the dark. In each germination test, four replications of 50 seeds were used. The 2 mm radicle protrusion was counted daily for the calculation of MGT, and cumulative germination and normal germination percentages (i.e. normally developed seedlings) were determined after 12 days.

The mean germination times were calculated on the basis of daily radicle protrusion counts using the following formula:

$$MGT = \Sigma (n.D) / \Sigma n$$

n= number of newly-germinated seeds at time D; D= days from sowing, beginning of tests;

Σ n= final germination (%)

Four sub-samples of two hundred seeds from each lot were stored hermetically at -20°C in airtight laminated aluminium packets. Before the samples were transferred to be stored at -20°C, the packets were left at 5°C overnight in order to reduce low temperature shock. Samples were taken from the storage after 0, 15, 30 and 60 days and standard germination tests (normal seedling percentages) were conducted at 20°C for 12 days in the dark. After taking the samples from -20°C, the packets were again left at 5°C overnight to defrost. Germination tests were then conducted as described above. A statistical analysis was performed using SPSS to compare the mean values of the untreated and treated and stored seed samples by using analyses of variance. Mean separation was made at the 5% level by the Duncan's multiple range test.

3. Results

Unstored and unprimed seeds in this study showed 80.6% mean germination, and 4.67 days of mean germination time (Tables 1 and 2). The range of the germination percentages of unprimed seed lots was 65% to 89%. These values went up to 74% to 95% after priming and before storage. The mean germination time ranged between 3.7 and 6.1 days in unprimed lots, and between 3.1 and 5.7 days in primed but unstored lots. The mean germination time in treated lots was reduced to 3.88 days (Table 2). The advance obtained from priming varied among the lots. The highest regarding germination, 9%, was obtained in cultivars 101-54 and 101-59. In some cultivars the treatment was not beneficial, as in 101-14, 101-11, and Asg-val-12. With respect to mean germination time, treatment reduced germination time significantly ($p<0.05$) in all cultivars except one (101-11, $p<0.05$) in which unprimed and primed seeds germinated in 4.6 and 4.5 days respectively.

Table 1. Variation in germination percentages of twenty control (C), primed and unstored (P) and primed and stored onion seed lots after 15, 30 and 60 days at -20°C. Means with the same letter in the same line are not significantly different at the 5% level.

Lots	Control (C)	Primed (P)	Primed and stored at -20 ◦C (days)		
			15 d	30 d	60 d
101-20	89 b	93 a	92 a	92 a	90 b
101-13	89 c	92 b	95 a	93 b	87 d
101-8	87 c	93 a	90 b	91 b	90 b
101-67	88 b	95 a	94 a	93 a	89 b
101-33	85 c	89 b	94 a	94 a	85 c
101-36	87 c	93 b	93 b	95 a	92 b
101-54	85 c	93 a	92 a	93 a	88 b
101-14	87 b	87 b	91 a	91 a	85 b
101-5	86 bc	85 cd	88 ab	89 a	83 d
101-7	80 d	83 bc	85 ab	87 a	82 cd
Valencia	75 b	81 a	80 a	81 a	79 a
Storm	78 b	81 a	83 a	85 a	75 c
101-11	85 b	84 b	90 a	91 a	82 b
101-1	73 c	81 b	88 a	86 a	80 b
101-54	65 c	74 b	80 a	81 a	75 b
Banka	76 b	78 b	82 a	81 a	73 c
101-54-4	70 b	77 a	78 a	75 a	70 b
101-59	85 b	94 a	95 a	95 a	86 b
Asg-val-12	72 ^{ns}	74 ^{ns}	74 ^{ns}	75 ^{ns}	72 ^{ns}
Asg-val-15	71 b	74 a	75 a	74 a	69 b
Mean	80.6 b	85.0 ab	86.9 a	87.1 a	81.7 b

Table 2. Variation in mean germination time of twenty control (C), primed (P) and unstored and primed and stored onion seed lots after 15, 30 and 60 days at -20C. Means with the same letter in the same line are not significantly different at the 5% level.

Lots	Control (C)	Primed (P)	Primed + stored (days)		
			15	30	60
101-20	4.6 d	3.5 b	3.5 b	3.3 a	3.9 c
101-13	5.0 d	3.5 b	3.6 b	3.1 a	4.1 c
101-8	3.9 d	3.5 b	3.4 ab	3.3 a	3.7 c
101-67	4.1 c	3.3 b	3.2 ab	3.1 a	4.1 c
101-33	4.1 d	3.1 a	3.3 b	3.2 ab	3.8 c
101-36	4.6 d	3.6 b	3.6 b	3.4 a	4.2 c
101-54	4.6 c	4.1 ab	4.1 ab	3.9 a	4.2 b
101-14	4.4 c	3.7 a	3.9 b	3.6 a	4.0 b
101-5	3.7 c	3.5 b	3.2 a	3.2 a	3.9 d
101-7	4.4 c	3.6 b	3.4 ab	3.3 a	4.3 c
Valencia	4.6 c	3.9 ab	3.8 a	3.7 a	4.1 b
Storm	4.8 c	3.8 a	4.2 b	3.7 a	4.7 c
101-11	4.6 b	4.5 b	4.0 a	4.1 a	4.8 c
101-1	5.1 b	4.4 a	4.4 a	4.3 a	4.4 a
101-54-5	6.1 d	5.7 b	5.9 c	5.1 a	5.0 a
Banka	4.9 d	3.7 b	3.9 c	3.5 a	3.9 c
101-54-4	5.5 e	4.3 a	5.0 d	4.5 b	4.7 c
101-59	4.5 d	3.4 b	3.0 a	3.0 a	4.0 c
Asg-val-12	4.6 c	4.3 b	4.2 b	3.8 a	4.8 d
Asg-val-15	5.3 d	4.2 b	3.8 a	3.8 a	4.8 c
Mean	4.67 b	3.88 a	3.87 a	3.64 a	4.3 b

Storing primed seeds at -20°C for 15 and 30 days reduced neither germination percentages nor mean germination time significantly (Figure 1, $p<0.05$) compared to primed and unstored seeds. The mean germination of 20 lots of primed unstored seed was 85%, and this value was 86.9% and 87.1% in of samples primed and stored for 15 and 30 days (Table 1). The mean germination time in primed and stored seeds was 3.88 days, and the value was 3.87 and 3.64 days for 15 and 30 days of storage (Figure 2). Seed lots primed and stored for 60 days showed significantly lower germination percentages and mean germination time than primed and unstored seeds ($p<0.05$). The value of primed seeds stored for 60 days differed from untreated control seeds ($p<0.05$) and seeds primed and stored for 15 and 30 days regarding both germination percentages and mean germination time (Figs 1, 2 and 3). When the individual seed lots were considered, it was seen that the germination percentages of 18 out of 20 of primed seeds stored for 15 and 30 days had equal or better performance ($p<0.05$) than treated and unstored seeds. This was reduced to nine seed lots in 60 days of storage (Table 1). These numbers were 14, 19 and 4 in mean germination time respectively (Table 2).

4. Discussion

This study showed that priming and storage of onion seeds had a beneficial effect compared to primed but unstored seeds until 30 days of storage at -20°C. Priming is a method that is used for many crop seeds in order to increase in germination percentages and rates under various environments (McDonald 2000; Khan et al. 2016). However, storage after priming gives contradictory results. The longevity of primed seeds is influenced by a complex interaction of factors including species, osmoticum, and water potential, duration of priming, dehydration and storage conditions (Parera and Cantliffe 1994). The quality of tomato seeds was maintained for several months at different storage temperatures (Argerich et al. 1989). Akers et al. (1987) reported that the effect of priming on parsley seeds was not reduced after eight months of storage. The advantages obtained from priming and storage at 10°C for about 12 months were similarly maintained in leeks and carrots (Dearman et al. 1987) and for 18 months of storage at 10°C in onions (Dearman et al. 1986). Germination of primed capsicum seeds was maintained over three years of storage at 25°C (Thanos et al. 1989). Sweet corn seeds stored for about 12 months at -80°C maintained quality (Chiu et al. 2002). However, primed and stored lettuce seeds deteriorated faster during storage than unprimed ones (Tarquis and Bradford 1992; Hill et al. 2007).

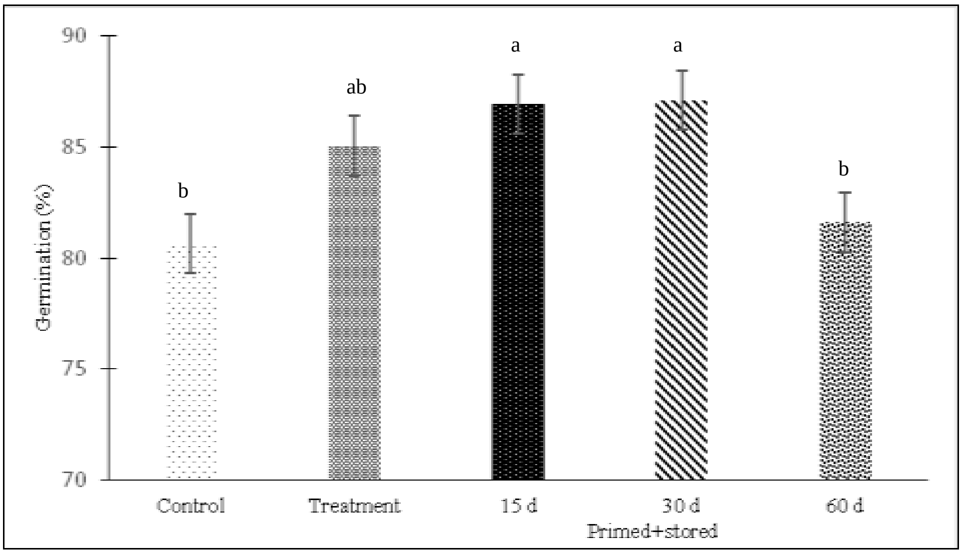

Figure 1. Mean normal germination percentages of twenty onion seed lots: control, primed and unstored and primed and stored for 15. 30 and 60 days at -20 °C. Bars on the tops of the columns are SEM. and letters indicate the difference among the treatments at the 5% level.

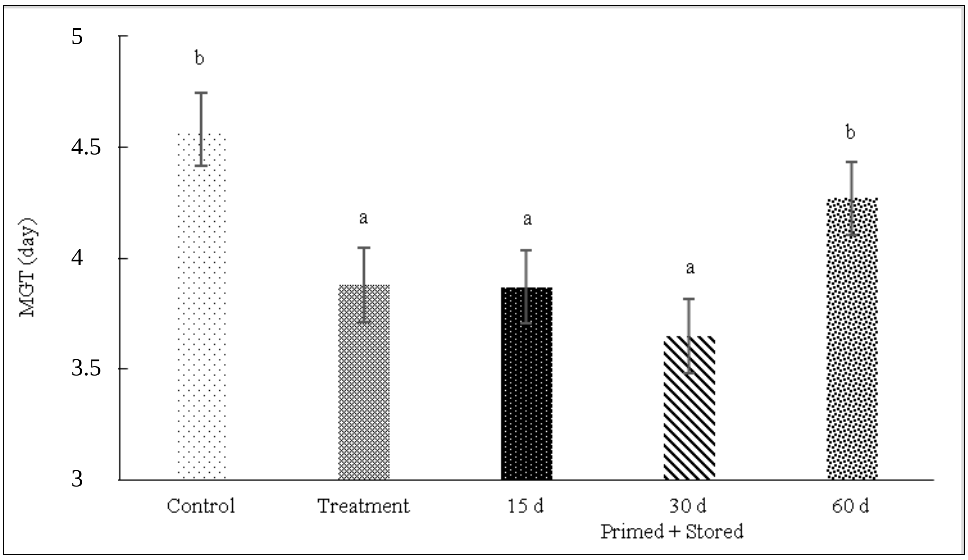

Figure 2. Mean of germination times of twenty onion seed lots: control (C), primed and unstored (T) and primed and stored for 15. 30 and 60 days at -20°C. Bars on the tops of the columns are SEM. and letters indicate the difference among the treatments at the 5% level.

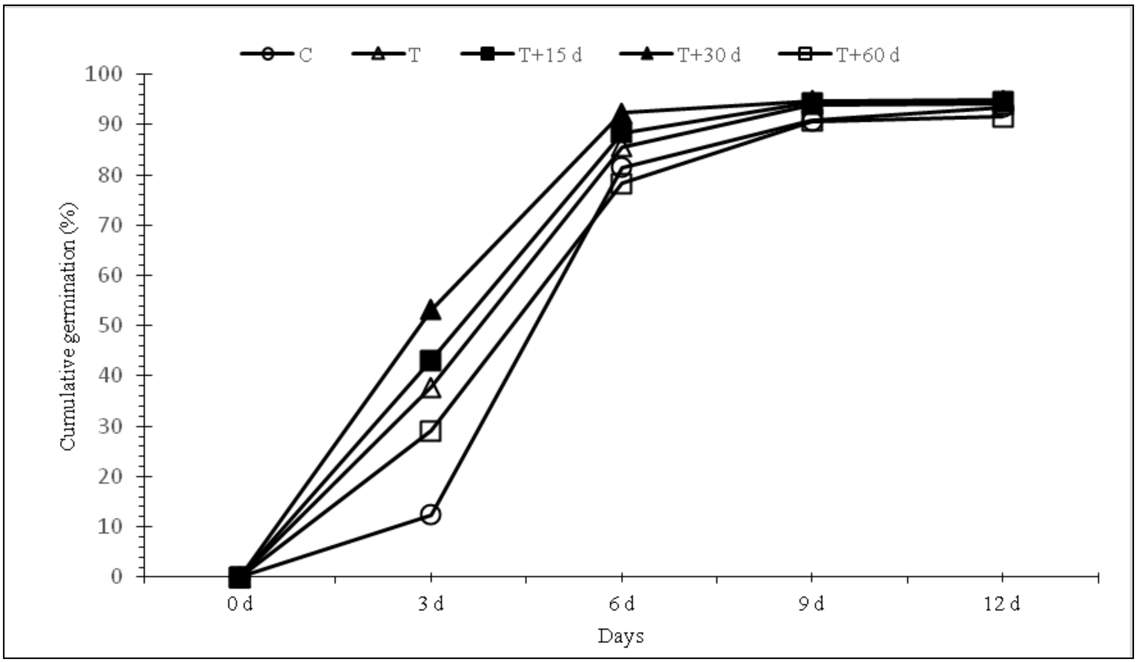

Figure 3. Cumulative mean germination percentages of twenty onion lots: control (C), primed and unstored (T) and primed and stored for 15 (T+15d). 30 (T+30d). and 60 (T+60d) days at -20°C.

These conclusions indicate that species differences as well as priming procedures play a role in the post priming storage effect. Furthermore, Chiu et al. (2002) showed that sweet corn seeds primed at a low temperature, 10°C, aged more slowly than those primed at 20°C. Hill et al. (2007) indicated that moisture was also important in primed lettuce seed. Primed lettuce seeds are more sensitive to storage when seed moisture is higher - 9 vs 6% - compared to unprimed seeds. The quality tests run after storage may also affect the results. When germination rate or accelerated ageing were used to compare primed and unprimed seeds, primed seeds had a shorter storage life. Also, when only germination percentages were used without vigour tests, there often appeared to be no difference between the storage life of primed and unprimed seeds (Olouch and Welbaum 1996). In our study, we found differences between primed and unprimed seed lots regarding germination and mean germination time. However, we did not conduct ageing tests, and so we could not test that hypothesis. This can be a further aspect that can be tested.

In our study, we tested 20 onion cultivars, 18 of which had significantly higher germination percentages than primed and unstored seeds after 15 and 30 days storage of -20°C (Table 1; Figure 3). When storage time was extended to 60 days, just nine seed lots showed an advantage. It seems to be that extended storage time may be hazardous for seed germination. Chiu et al. (2002) stored sweet corn seeds at a much lower temperature of -80°C, and germination was as good as that of primed and unstored seeds after 12 months. This obviously shows that the effect may vary not only between species but even between seed cultivars (Khan et al. 2016). The basic physiological fact about priming is that its effect can vary not only between species but also between lots. The chemical contents of the seeds may be one reason why seed species behave differently after the storage of primed seeds. For instance, sweet corn contains a high proportion of sugar while onion and lettuce are oily seeds (Walters et al. 2005). It has been long known that highly oily seeds are more prone to ageing in storage (Roos 1989).

Primed seeds are described as having 'high seed quality with low storability' in some cases. Therefore, the best practice may be sowing seeds immediately after treatment. However, in some cases, seeds are not sown for environmental reasons or because sowing conditions in the field, such as temperature and soil conditions, are unsuitable. Thus, storage may be a necessity. In such cases, our results indicate that onion seeds can be stored for about 30 days at -20°C. In storage at such low temperatures, the key point is seed moisture. Sub-frozen temperatures can be damaging due to freezing injury in seeds with high moisture contents. For this reason, seed moisture should be reduced. In seed gene banks, it is suggested that seeds should be stored at -20°C with 5-7% seed moisture content, which is an internationally accepted value for long term storage in order to get the minimum deterioration in storage (Walters et al. 2005). We dried seeds after priming in this study to about 7% so as to avoid the occurrence of freezing injury. In this way, the seeds were easily to handle and use for sowing methods such as machine sowing.

One other aspect that should be considered in storage at -20°C is the rate of freezing. We kept seeds overnight for about 24 hours at 5°C before freezing them at -20°C. We did not put the packets straight into sub-zero temperature. Similar precautions were taken while taking the samples out of the store after storage. The packets were kept at 5°C overnight before conducting seed germination tests. Such a method is suggested as a precaution in seed gene bank methodologies to avoid freezing damage, particularly to cell membranes.

5. Conclusion

Storing primed seeds at -20°C over a couple of months may not be easy for a large proportion of seeds. However, it can be an alternative methodology for certain high value seeds such as primed hybrids, rootstock seeds, breeder lines and samples. Moreover, this method can be an alternative storage technology for inherently short-lived species including onions.

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