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The analysis of germination of two types of *Momordica charantia* L. seeds in different media after different storage durations

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Abstract
Momordica charantia L. is a member of Cucurbitaceae can be use as rootstock for obtain seedlings againsts some abiotic and biotic stress factors. For these reasons, the aim of this study was to evaluate effect of origin of the plant types, germination media and seed storage duration on seed germination and seedling rates. The two different plant types (A and B) belonging to non-commercial gardens from two different districts located 30 km from each other in Kocaeli City were tested in 3 seed germination media (paper towel, petri dishes and sand) after 0, 90, 180 and 270 days storage under dark-room condition. First and last germination days, first and last leaf appearance days, germination and seedling rate were monitored. The study designed as Completely Randomized Design replicated thrice and after statistical analyzes, analysis of variance with Duncan Multiple Range Test method at $P< 0.05$ was performed. Considering the whole experiment first germination days changed between 4th-5.7th and last germination days changed between 4th-13.7th days. There is no difference between the plant types in the parameter at all the three media. Maximum germination and seedling ratio were observed at type-A in the all media. In petri, the germination and seedling ratio parameter decreased gradually with storage duration although storage duration showed no differences in paper towel or sand in the germination and seedling parameters. Optimum seedling ratio of *Momordica charantia* was observed when sand media were used at ratio of 99.2% in type-A and 88.35% in type-B in averages at all the storage durations under the study condition.

Keywords: *Momordica charantia* L., bitter gourd, germination, seed storage, rootstock

Introduction
Momordica charantia L. is a member of the Cucurbitaceae family and can be used as rootstock to prevent some of biotic and abiotic diseases for intensively culturable other cucurbits at growth stages [1-4] besides of edible and remedy properties for human consumption and human diseases treatments [5-7]. At the same time the plant has natural plant protection chemicals in different parts of its [8-10]. The effect of seed origin and seed storage time on plant growth has received considerable attention in many studies [11-14]. Germination media are also show differences on testing germination capacity on plant seed [15-17]. Because of high demand and continuosly cultivation of cucurbits, sometimes the plants face to growth under unfavorable conditions in some part of countries in the world as too cold, wet, dry, low-light along the year [18] and some of the undesirable situations can be eliminated by grafting. The germinative and developmental capacity of the seedling of rootstock has an important effects on obtaining health grafted seedling in vegetables. The aim of the study is to reveal whether the germination media, seed origin and storage time have an effect on the germination of seeds of *Momordica charantia* L. types that have the potential to be used as rootstocks.

Material and Method
Plant material and media preperation: This experiment was performed to investigate the effect of seed origin, germination media and storage duration on seed germination and seedling rate under laboratory condition at Kocaeli University, Agriculture Faculty. In order to fulfil this aim fruits of *Momordica charantia* L. were obtain from two different rural areas at two different districts (İzmit District; Type-A and Karamürsel District; Type-B) located 30

km from each other of Kocaeli City in Türkiye. The fruits, belonging to non-commercial gardens, and only from one main plant each, were picked in October, 2019 (Figure 1a, 1b). The day after fruit picking, pericarp and aril were removed (Figure 1c, 1d) and seed were taken into germination media for 0 day storage study. For the studies on 90, 180 and 270 days storage duration, the remaining seeds were kept in paper bag under room temperature in darkness until they are being placed the similar media and conditions. The three germination media were paper towels, petri dishes and sand (by wrapping seeds between two layers of paper towel, by layering seeds between two layers of filter paper at petri dishes and by spreading on the sand surface respectively). 100 witness seeds were kept under the same storage condition and were weighted to determine weight lost just before the all four seed planting time. According to the mean of the weighing results one seed was;
0,21712 g in Type-A and 0,18684 g in Type-B at 0. day;
0,21489 g in Type-A and 0,18415 g in Type-B at 90. day,
0,21404 g in Type-A and 0,18369 g in Type-B at 180. day, and
0,21332 g in Type-A and 0,18363 g in Type-B at 270. day.

Experiment: After the seed placement and watering with same quantity, the media were protected with plastic, transparent soft bags for protect moisture. The media kept under 25^{0}C, 16/8 h day/night photoperiod. The day after the seed placement, first and last germination day (calculated from at least 1 cm long rooted seed), first and last leaf appearance day (calculated from when true leaf slightly visible) noted daily. At the end of the study total germination (calculated from at least 1 cm long rooted seed) and succeful seedling capacity rate (calculated from at least 1 cm rooted seed with visible true leaf) of the plants were noted.

Statistical analysis: The study was designed as Completely Randomized Design replicated thrice and each replicate contains 10 seed (2 seed types x 4 storage duration x 3 germination media x 3 repeat=720 seeds in total). After statistical analysis, analysis of variance with Duncan Multiple Range Test method at $P< 0.05$ was performed.

Fig 1: *Momordica charantia* L.; a) Fruit sample of Type-A, b) Fruit sample of Type-B, c) Cross section of fruit sample in Type-A, b) Seed samples; Type-A in left and Type-B in right (seeds in aril in the top, used seeds without aril in the middle, longitidunal bisected seeds in the bottom

Results and Discussion

According to the data the first germination days changed between 4th-5,7th days in both seed types, in all storage duration and at all used media. Only at paper towel, there are differences between storage duration in both of the seed types. Besides that, the last germination days that changed between 4th-13,7th days in whole experiment, showed differences at paper towel in Type-A, at petri in Type-B and at sand both Type-A and B. Although there are no differences in the types, there are some findings that when storage duration is longer, the last germination days shorten at paper towel for Type-A and at sand for Type A and B (Table 1).

The first and the last leaf appearance days changed between 6th-10,3rd days and 8,3rd-15,7th days respectively in all study factors. According to the results that changing is related with interaction of storage duration and the used media than between plant types in avarages (Table 2) except sand for last leaf appearance day the medium showed that longer storage caused early leaf appearance in Type-A statistically and in Type-B numerically.

The total germination and the seedling capacity rates changed between 66,7%-100% and 33,3%-100% respectively in all media, plant types and storage durations. In total at paper towel and sand showed differences in seed types in seedling rate and only sand showed germination rate differences in seed types (Table 3). When petri was used, both the germination and seedling rate decreased with the longer storage duration at both of two seed types. But results from paper towel and sand do not confirm this findings. According to the petri observations, the reason for this may be due to the increase in microorganism activities as the longer storaged seed that cause seed damping-off in petri with lack of air than other used media.

Kanwar *et al*. [19] also studied with paper towel and sand method at their first step of study on *M. charantia* and found that 4th day first germination occured. The used media and the first germination day are compatible with our study. Barraza-Alvarez [20] also studied on *M. charantia* germination in petri and found 62% germination in control and 76% in 24 hours-water soaked treatment. Similarly in averages of storage durations, petri results were the lowest in germination and seedling rate in both seed types in our study. Ghosh *et al*. [22] and Singh *et al*. [23] emphasized that *M. charantia* has broad genetic variability of the genotypes and strains that used in their studies for nearly all the measured characters. This results supports the findings from some media of our study. Most seeds belonging Cucurbitaceae are orthodox and desiccant tolerant at maturity [24] and orthodox seeds have long storability [25]. Our study supported the general concept with the used storage durations. There were no differences in germination and seedling rates depending on the storage durations except petri. In addition Nienhuis and Lower [26] also found no

storage duration effect on germination of *M. charantia* along 30 weeks, that were 91.5% at 25^{0}C that we also used this temperature. The data were very close with the findings of our study.

Conclusion

The different media, the different storage duration and the different seed types showed that they are changegable parameter in correlation with others. But the results indicated that the earliest germination occured in 4th and the latest one in 13,7th days. The lowest-highest germination rates were found 66.7%-100% and the lowest-highest seedling rates were found 33.3%-100% in all the used media, the seed types and the storage durations. Unless the right medium is chosen, it is not possible to reach a definite judgement about the germination days, leaf appearance days and germination and seedling rates of seed types and storage durations. The results from the sand medium seems more reliable for obtain rootstocks for grafting. According to the data from sand; last germination day occured quickly with the longer storage period. Additionally storage period had no effect on germination and seedling rates with the highest results at sand among all used medium. The chosen seed types had effect on the two parameter in storage duration averages at the sand medium. As a conclusion sand medium is more reliable for reach data on some seedling features and 270 days along storage period had no effect on seedling rate and there are statistically differences between seed types in averages on germination and seedling rates for *Momordica charantia* L. that can be a choice as a rootstock for healty cucurbit growth against abiotic and biotic problems.

Table 1: Mean days of first and last gemination of *Momordica charantia* L. after 30 days observation at different germination medium and after different storage durations

Germination Medium	Seed Storage Duration (days)	First Germination Day		Last Germination Day	
		Initial Plant Types		Initial Plant Types	
		Type-A	Type-B	Type-A	Type-B
Paper towel	0	4,7 b*	4,7 b*	10,3 b*	8,7**
	90	4,0 a	4,0 a	7,3 ab	8,7
	180	4,0 a	4,0 a	7,3 ab	6,7
	270	4,0 a	4,0 a	6,0 a	7,0
	Average	4,2***	4,2***	7,7***	7,8***
Petri	0	5,3**	5,7 b*	10,7**	10,0 ab*
	90	5,0	4,3 a	11,3	12,0 ab
	180	5,0	4,3 a	9,7	9,0 a
	270	4,0	4,0 a	9,7	13,7 b
	Average	4,8***	4,6***	10,4***	11,2***
Sand	0	4,7**	4,3 a*	8,3 b*	10,6 b*
	90	4,0	4,0 a	5,7 ab	10,0 b
	180	5,0	5,0 b	8,0 b	12,3 b
	270	4,0	4,0 a	4,0 a	4,3 a
	Average	4,4***	4,3***	6,5***	9,3***

*Lower case indicates significantly differences in only one used medium and only one seed types for all storage durations, **indicates non-significantly differences in only one used medium and only one seed types for all storage durations, ***indicates non-significantly differences between averages of storage durations between the seed types in only one used medium.

Table 2: Mean days of first and last leaf appearance of *Momordica charantia* L. after 30 days observation at different germination medium and after different storage durations

Germination Medium	Seed Storage Duration (days)	First Leaf Appearance Day		Last Leaf Appearance Day	
		Initial Plant Types		Initial Plant Types	
		Type-A	Type-B	Type-A	Type-B
Paper towel	0	8,0 b*	8,3 b*	12,3**	11,0 a*
	90	7,0 a	7,0 a	11,7	11,0 a
	180	8,3 b	8,0 b	11,3	13,0 b
	270	7,7 ab	8,0 b	13,3	14,3 b
	Average	7,8***	7,8***	12,2***	12,3***
Petri	0	9,3 ab*	10,0**	15,7**	14,7**
	90	9,0 a	9,0	14,3	14,0
	180	10,3 b	8,7	12,3	13,6
	270	9,0 a	9,7	13,3	14,7
	Average	9,4***	9,4***	13,9***	14,3***
Sand	0	7,7 b*	7,7 bc*	10,0 ab*	14,0**
	90	6,0 a	6,0 a	9,3 ab	12,7
	180	7,7 b	8,3 c	11,3 b	14,3
	270	6,3 ab	7,0 b	8,3 a	10,7
	Average	6,9***	7,3***	9,8 A****	12,9 B****

*Lower case indicates significantly differences in only one used medium and only one seed types for all storage durations, **indicates non-significantly differences in only one used medium and only one seed types for all storage durations, ***indicates non-significantly differences between averages of storage durations between the seed types in only one used medium, ****capital letters indicates significantly differences between averages of the seed types of storage durations in only one used medium.

Table 3: Mean rates of germination and seedling of *Momordica charantia* L. after 30 days observation at different germination medium and after different storage durations

Germination Medium	Seed Storage Duration (days)	Germination Rate		Seedling Rate	
		Initial Plant Types		Initial Plant Types	
		Type-A	Type-B	Type-A	Type-B
Paper towel	0	100,0**	93,3**	96,7**	86,7**
	90	96,7	83,3	96,7	80,0
	180	100,0	96,7	90,0	76,7
	270	96,7	96,7	93,3	86,7
	Average	98,4***	92,5***	94,2 A****	82,5 B****
Petri	0	96,7**	90,0**	90,0 a*	76,7 a*
	90	90,0	83,3	86,7 a	70,0 a
	180	86,7	80,0	36,7 b	43,3 b
	270	76,7	66,7	50,0 b	33,3 b
	Average	87,5***	80,0***	65,85***	55,9***
Sand	0	100**	90**	100**	83,3**
	90	100	86,7	100	86,7
	180	96,7	86,7	96,7	86,7
	270	100	96,7	100	96,7
	Average	99,2 A****	90,0 B****	99,2 A****	88,4 B****

*Lower case indicates significantly differences in only one used medium and only one seed types for all storage durations, **indicates non-significantly differences in only one used medium and only one seed types for all storage durations, ***indicates non-significantly differences between averages of storage durations between the seed types in only one used medium, ****capital letters indicates significantly differences between averages of the seed types of storage durations in only one used medium.

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