Original Research Article

GERMINATION AND LONGEVITY OF SOME COWPEA CULTIVARS AFFECTED BY SINGLE AND MIXED VIRUS INFECTIONS IN NGER STATE, NIGERIA

ABSTRACT

A field trial was carried out at the Teaching and Research Farm of the Faculty of Agriculture, Ahmadu Bello University (ABU), Zaria, Mokwa Station (09^{0}211′N and 5^{0}135′E, 201 m.a. s. l.) in 2017. Mokwa is situated in the Southern Guinea Savanna agro - ecological zone of Nigeria. The trial was conducted to assess the response of twenty five cowpea cultivars to single and mixed infections with *Blackeye cowpea mosaic virus* (BlCMV) and *Cowpea mottle virus* (CPMoV) and their effects on seed quality. Seed viability test was determined in the Laboratory of the Department of Crop Production, Federal University of Technology, Minna, Nigeria. Four independent trials were conducted simultaneously, for single and mixed infections. The field was cleared, ploughed, harrowed and ridged at 0.75 m apart, then marked out into plots and set up in a randomized complete block design (RCBD), replicated three times. The test seeds were dressed with Apron – star (methylthiuram + metalaxyl + carboxin) at the of rate 3.0 kg seed per 10 g of the fungicide. Three cowpea seeds of each cultivar were sown at intra and inter– row spacing of 0.30 × 0.75 m along the ridges and later thinned to two per stand at 2 weeks after sowing (WAS). For the single virus infection, seedlings of the twenty five cultivars were inoculated at 10 days after sowing (DAS) while the mixed virus infections seedlings were inoculated at 10 and 17 DAS. Results showed that all the cowpea cultivars were susceptible to single and mixed infections of the two viruses but were seemingly different to some extents. Viability of seeds from single infection with CPMoV was slightly reduced in some instances, but some seeds' viability was not much affected. Test of accelerated ageing for four weeks showed that seed vigour was seriously impaired as compared to the other three virus treatments.

Keywords: *Blackeye cowpea mosaic virus*, *Cowpea mottle virus,* Cowpea seeds, Seed quality, seed viability.

INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp) is one of the most ancient crops known to man (Davis *et al*., 1999). Its origin and domestication occurred in Southern Africa and was later moved to East and subsequently was developed mainly in the farms of the African Savanna and Asia (Langyintuo *et al.*, 2003, Gómez, 2012). Today, it is widely adapted and grown throughout the world but Africa predominates in production with Nigeria being the largest producer (Kormawa *et al*., 2002).

Cowpea is a major staple food crop in sub-Saharan Africa, especially in the dry savanna regions of West Africa (Dugje *et al*., 2009,). The seeds are a major source of plant protein and vitamins for man, feed for animals, and also a source of cash income (Davis *et al.*, 1991, Phillip *et al*., 2003, Mamiro *et al*., 2011, Adino *et al*., 2018, da Silva *et al*., 2018).

The young leaves and immature pods are eaten as vegetables (Dugjie *et al*., 2009). It has been estimated that the annual world cowpea crop is grown on 12.5 million hectares, and the total grain production is 3.9 million tonnes (FAO, 2016). More than 8 million hectares of cowpea are grown in West and Central Africa (Langyintuo *et al.*, 2003). Nigeria is known to be the largest producer with 4 million hectares which accounts for 45 % of the total on 1.15 mllion hectares annually (Kormawa *et al*., 2002, Dugjie *et al*., 2009). Other producers are Niger, Mali, Burkina Faso and Senegal (Gómez, 2012).

The major cowpea producing areas in Nigeria include Niger, Kwara, Kaduna, Borno, Taraba and Yobe States in the northern part while Oyo, Ogun and Ondo also produce appreciable quantities in the southern part of the country (IITA, 2013).

Virus diseases are considered to be a major limiting factor for the production and productivity of legumes in the tropical and sub-tropical countries (Bashir *et al*., 2000, Vincent *et al.,* 2014, Palanga *et al*., 2016). Out of more than 20 viruses reported on legumes from different parts of the world, (Kareem and Taiwo, 2007, Vincent *et al.,* 2014, Palanga *et al*., 2016), nine are known to infect cowpea naturally in Nigeria. One of these, *Blackeye cowpea mosaic virus* (BlCMV) (genus *Potyvirus*, family *Potyviridae*) was first reported on cowpea in the U.S. in 1955 (Alegbejo, 2015). It is distributed in all ecological zones and cowpea- growing areas of Nigeria. Local symptoms appear as large reddish lesions that spread along the veins, while systemic symptoms appear as severe mottle, mosaic, vein-banding, veinal chlorosis, distortion and stunting of the plant. Disease symptoms vary with virus strain and host cultivar. Incidence varies from 1-40 % on farmers' fields (Najar *et al*., 2011, Makkouk *et al*., 2014) and yield losses due to the virus vary from 10-85 % on individually infected plants and vary with time of sowing (Taiwo *et al*., 2007)

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Cowpea mottle virus (CPMoV)(genus *Gammacarmovirus*, family *Tombusviridae*) is a positive sense single-stranded RNA, unipartite, isometric virus, 30 nm in diameter (Alegbejo, 2015). The pathogen is distributed in all ecological zones of Nigeria, particularly in the riverine areas of the middle belt which has a Southern Guinea Savanna climate and where a lot of bambara groundnut is grown (Reddy and Devi, 2010). Infected plants display severe mosaic, mottling or bright yellow mosaic. Leaf distortion and reduction in leaf size sometimes lead to a witches' broom appearance in cowpea (Bhat *et al.,* 2011).

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Seed-borne viruses are important source of diseases at the beginning of production even at low rates of seed transmission (Kareem and Taiwo, 2007). In addition, seed-borne viruses can aggravate other transmission methods and cause disease to spread rapidly (Taiwo *et al*., 2007).

Seed-borne and seed transmitted viruses are also damaging to cowpea productivity owing to inherent primary inoculum and potential for their wide dispersal (Arogundade *et al*., 2010). Information on the possibility of seed transmission in virus infected cowpeas will be valuable to numerous cowpea farmers. Again, information on germination of infected seeds and survival of resulting plants, virus disease progress during the growing season, magnitude of yield loss and amount of infection in harvested seeds in replicated field experiments is required to establish acceptable threshold levels of seed-borne infections. Thus, the present study is essential to develop preventive and management measures for cowpea virus diseases in Niger State. Therefore, this work is aimed at examining the effects of virus infections on cowpea seed viability and seed quality.

MATERIALS AND METHODS

Field trial

The was conducted during the 2017 wet session at the Teaching and Research farm of the Faculty of Agriculture, Ahmadu Bello University (ABU), Mokwa Station ($09^{o}211^{'}$N and $5^{o}135^{'}$E, 201 m. a. s. l.). Mokwa is situated in the Southern Guinea Savanna agro - ecological zone of Nigeria. The site used for the research was under continuous cropping with maize and beans between 2012 till the commencement of the study.

Screening site, treatments and experimental design

Four independent trials were conducted simultaneously, for single and mixed infections of the two most common viruses in the study area. In each trial, 25 cowpea cultivars namely Ife Brown, IT90K – 277 – 2, IT96D – 610, IT97K – 499 – 35, IT97K – 568 – 18, IT97K – 573 – 2 –1, IT98K – 205 –M8, IT98KD – 288, IT99K – 316 – 2, IT99K – 377 – 1, IT00K – 901 – 5, IT03K – 337 – 6, IT04K – 267 – 8, IT04K – 291 – 2, IT04K – 321 – 2, IT04K – 332 – 1, IT06K – 124, IT06K – 137 – 1, IT07K – 211 – 1 – 8, IT07K – 222 – 2, IT07K – 243 – 1 – 10, IT07K – 251 – 3 – 3, IT07K – 292 – 1 – 10, IT07K – 299 – 6 and IT07K – 318 – 33) constituted the treatments. The cultivars were photosensitive and high yielding under virus free conditions. The field was cleared, ploughed, harrowed and ridged with tractor at 0.75 m apart then marked out into plots. Each cultivar was evaluated in 0.375 m ridge wide, 3 m long and 0.75 m apart giving a total plot size of 18.75 m per replicate. The trial was arranged as randomized complete block design (RCBD) and replicated three times giving a total land area of 900 m^2.

Source of inoculum and multiplication

The ***Blackeye cowpea mosaic virus*** (BICMV) and ***Cowpea mottle virus*** (CPMoV) isolates used were obtained from the Department of Crop Production, Federal University of Technology, Minna, Nigeria. The virus isolates were extracted by grinding 1g/ml of each isolate in extraction buffer containing 0.1M sodium phosphate dibasic, 0.1M potassium phosphate monobasic, 0.0lM ethylene diamine tetra acetic acid and 0.00lM-cystine per litre of distilled water using a pre-cooled sterilized mortar and pestle as described by Kumar (2009). Two microlitres of β-mercapto-ethanol were added to the extract just before use. Thereafter, cowpea seedlings were infected with BICMV and CPMoV inoculum at 10 days after sowing (DAS) by rubbing the virus extracts on the upper surface of the leaves that was dusted with carborundum powder of 600-mesh.

Comment [Anon1]: Complete nemaes were already defined in the introduction

The leaves of inoculated plants were rinsed with sterile distilled water and symptomatic cowpea leaves were collected from the infected plants at 3 weeks after inoculation (WAI) and used for inoculation during the main experiment. The leaves were preserved at room temperature in airtight vial bottles on silica gels covered with a thin layer of non-absorbent cotton wool.

Land preparation , planting, seed treatment and inoculation

The study site was cleared of the previous plant remains with the tractor and ridged in the second week of August, 2017. The cowpea seeds were dressed with Apron – star (methylthiuram + metalaxyl + carboxin) at the of rate 3.0 kg seed per 10 g sachet of the fungicide to protect them against soil borne pathogens. Three treated cowpea seeds of each cultivar were sown at intra and inter– row spacing of 0.30 × 0.75 m along the ridges and later thinned to two per stand at 2 weeks after sowing (WAS).

The BICMV and CPMoV infected cowpea leaves previously preserved on silica gels were used for inoculation. For the single virus infection, seedlings of the twenty five cultivars were mechanically inoculated singly with BICMV or CPMoV at 10 days after sowing while for the mixed virus infections, seedlings were inoculated at 10 and 17 DAS. Weeds were manually controlled through hand weeding at 4 and 6 weeks after sowing. Insect pests were controlled by spraying D-D force (Cypermethrin plus Dimethoate) and pods were harvested at physiological maturity. The pods were processed and packaged for seed viability and quality assessments in the laboratory.

Assessment of Virus Infection on Seed Quality

Seed lots from the various virus treatments were subjected to seed quality test as follows;

Germination and longevity of seeds of all the virus treatment combinations were determined by germination test after harvest and at four weeks of storage respectively in the Laboratory of the Department of Crop Production, Federal University of Technology, Minna. Twenty five seeds were placed in distilled-water- moistened filter paper lined in Petri-dishes and laid in three replicates. The filter paper in the petri-dishes was kept moist as found necessary.

The petri-dishes were arranged inside the seed germination chamber and germination counts were taken at 1, 2, 3, 4 and 5 days after sowing. Seeds were considered germinated when the tip of the radicle had grown free from the seed coat as described by El Balla *et al*. (2011). Germination percentage (GPCT) was calculated as follows:

GPCT= Total number of seedlings that emerged on the final day × 100

Total number of seeds planted

Cowpea seeds were also subjected to accelerated ageing tests at two and four weeks as described by El Balla *et al*. (2011) for vigour determination. The seeds from all the treatments were stored in open plastic plates and arranged inside an incubator at 35 ^{o}C and 86 % relative humidity. This was aimed at accelerating the ageing of the seeds so that the relative longevity of the seed samples could be determined. Twenty five seeds from each treatment that were artificially aged in three replications were counted and placed on the layer of distilled water moistened-filter paper in petri-dishes over a wire mesh screen inside a growth chamber at 30 ^{o}C. Germination count was taken as described above.

Data analysis

Data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS, 2008) to verify if there were significant differences among the cultivars. Significance was determined at 5 % level of probability. Where the *F*-test ratio was significant, means were separated using Student-Newman-Keuls (SNK) test.

RESULTS

Effects of single and mixed virus infections on seed viability and quality

The study revealed significant impairments in germination before and after four weeks of storage of the 25 cowpea cultivars in both single and mixed infections of the test viruses. The variation in seed germination of the cowpea cultivars with respect to virus infections is presented in Table 1. Prior to the storage of seeds, the difference between the lowest and highest mean value for seed germination was wide and significant ($p<0.05$).

Seed germination percentage varied from 77.4 to 99.7 % for the BICMV infected cultivars, 77.4 to 98.7 % for CPMoV infected cultivars, 74.8 to 98.5 % for BICMV + CPMoV infected cultivars and 78.6 to 98.5 % for CPMoV + BICMV inoculated cultivars (Table 1).Seeds obtained from IT97K-568-18, IT04K-332-1 and IT07K-292-1-10 cowpea cultivars infected with BICMV had significantly ($p<0.05$) higher germination percentage of 99.7 which was statistically similar to 97.6 and 97.3 % germination obtained from seeds of cultivars IT07K-243-1-10 and IT03K-337-6 respectively. Seeds from cultivars IT90K-277-2, IT07K-211-1-8 and IT06K-124 had germination values of 94.7, 94.3 and 93.7 % respectively which were not significantly different among each other. Seeds of cultivars IT07K-251-3-3 and IT07K-222-2 had 92.3 and 92.5 % germination values respectively which were statistically similar while seeds from the remaining cowpea cultivars had germination percentages ranging between 77.4 and 91.3.

Furthermore, seed germinability of 98.7 % was highest in IT90K-277-2 with CPMoV infected cowpea seeds which was not significantly ($p> 0.05$) different from seeds obtained from cultivars

IT04K-332-1 with 98.5 %, IT07K-243-1-10 with 98.4 %, IT04K-267-8 with 98.2 % and IT96D-610 with 97.7 %, while significantly lowest seed germination percentage of 77.4 was recorded in seeds of cowpea cultivar IT07K-292-1-10 (Table 1). On the other hand, co-infections of cowpea seeds significantly (p<0.05) affected seed germinability across the cowpea cultivars investigated. The BICMV + CPMoV infected IT04K-332-1 recorded the highest germination percentage of 98.5 % than all the other cultivars, whereas cultivars IT96D-610 and IT97K-499-35 recorded 97.6 % each. Seeds of cultivars IT07K-292-1-10 and IT97K-573-2-1 recorded 96.0 and 94.8 % germination respectively, while seeds of cultivar IT07K-222-2 recorded the lowest germination percentage of 74.8.

Seeds obtained from cultivar IT97K-568-18 infected with CPMoV + BICMV recorded the highest germination percentage of 98.5 before storage which was not significantly (p> 0.05) different from 97.3 % obtained from seeds of cultivar IT99K-316-2. Next to these with high germination percentage of 96 were seeds obtained from cultivars IT90K-277-2, IT96D-610, IT98K-205-M8, IT98KD-288, IT04K-332-1 and IT07K-222-2 whereas the significantly lowest germination percentage of 78.6 was recorded in seeds of cowpea cultivars IT04K-321-2 and IT07K-211-1-8 (Table 1).

Similarly, the difference between the lowest and highest percentage mean values for the longevity test was wide and significant (p<0.05) when seeds were stored for four weeks. Significantly, the highest germination percentage of 77.9 was recorded in seeds of BICMV infected Ife Brown followed by cultivars IT90K-277-2, IT00K-901-5 and IT96D-610 with 76.6, 70.6 and 70.3 germination percentages, respectively. Seeds of cultivars IT97K-568-18, IT07K-292-1-10 and IT07K-299-6 recorded germination percentage values of 69.5, 64.4 and 62.1 %, respectively whereas the least germination value of 46.6 % was obtained from seeds of cultivar IT06K-124.

The mean value for accelerated ageing germination (AAG) of CPMoV infected cowpea cultivars showed that seeds of cultivar IT98K-205-M8 recorded 70.6 % germination. This was closely followed by seeds of Ife Brown with 69 % while germination of 68, 66.8 and 66.5 % were obtained from cultivars IT90K-277-2, IT03K-337-6 and IT96D-610, respectively. The germination capacity of 64 % was recorded from seeds of cultivars IT99K-316-2 while IT07K-299-6 and the remaining cultivars recorded percentages ranging from 53.4 to 62.7 % (Table 1).

For the mixed infection treatments, germination value of 58.6 % was obtained from cultivars IT90K-277-2, IT06k-124 and IT07K-292-1-10 BICMV + CPMoV infected cowpea cultivars. This value was significantly (p<0.05) higher than the values obtained from seeds of the other cultivars. Seeds obtained from cultivars IT98K-205-M8, IT97K-499-35, IT06K-137-1 and IT07K-211-1-8 recorded germination of 56.5, 55, 54.5 and 53.4 % respectively. Seeds of cultivars IT96D-610 and IT00K-901-5 recorded similar germination percentage of 52 while the remaining cowpea cultivars recorded germination percentages of between 44.0 and 50.6.

Also, seed germinability of 57.3 % was highest in cultivar IT07K-292-1-10, CPMoV + BICMV infected cowpea seeds which was statistically (p>0.05) similar to the performance of seeds of cultivar IT97K-499-35 with 56 %. Seeds of cultivars IT04K-267-8 and IT07K-222-2 recorded germination of 54.6 and 53.7 % respectively, while cultivars IT96D-610 and IT04K-291-2 had germination values of 52 % which did not differ from each another. The lowest percentage of 31.6 was recorded in seeds of cowpea cultivar IT99K-377-1 (Table 1).

DISCUSSION

Germination and longevity are two major indices used for determining the performance capability of seed lots (Ibrahim, 2015). Seed quality and viability are influenced by the environment where seed is produced (Luzuriaga *et al*., 2006, Penfield and McGregor, 2017, Massimi, 2018). Pathogens such as viruses, nematodes, fungi, and bacteria among others are integral components of the environment of any seed crop; failure to effectively manage their competition could mean zero harvest (Adesina *et al*., 2012) as is found in this study. However the imperative of understanding the impact of virus management strategies and management for quality seed production arises from the paucity of information on the agronomy of seed production (Adesina *et al*., 2012), more so that seed production efforts are judged on the basis of the quality of the produce rather than its quantity.

The result of the present study has established a clear negative influence of virus infection on cowpea seed quality and viability and that the differential ranking of the virus infection treatments in the different seed quality test is an indication of the response of the developing seeds on the mother plant to competing virus infection situations. Differences in time of flower initiation, pod setting, seed formation and maturity to virus infections are critical factors to tropical farming. The results obtained from this study have shown that there was a variation in germination percentage before and after four weeks of storage which is a measure of seed viability and longevity as reported by Penfiel and MacGregor (2017).

When seed that has these traits is sown in the field for production, it exhibits a wide variation in performance after sowing due to the differences in its quality (Adesina *et al*., 2012, Ibrahim, 2015). Since the seeds did not ripen at the same time amongst the virus treatments across the test cowpea cultivars, variation in seed germination and longevity due to age at harvest was inevitable confirming the report by Singh (2014).

It is known that cowpea seedlings are susceptible to virus infection at different stages of development (Agrios, 2005). This is supported by the differential responses of cowpea seeds harvested from the different virus treatment seed lots in the present study. The initial general high germination percentage recorded in seeds of all the treatment combinations in this study is an indication that the seeds did not exhibit dormancy. This is contrary to what is known with most vegetable seeds when freshly harvested as reported by other workers (Khalil *et al*., 2012; Paylan *et al*., 2014 and Penfield and MacGregor, 2017). The rapid germination witnessed also showed that the effects of the viruses on the seeds were not severe enough to impair germination just as was reported by Anjorin and Mohammed (2014). Mandhare and Gawade (2010) similarly reported that though seeds obtained from mosaic infected beans exhibited high seed germination at harvest, a significant sharp decline in germination percentage of the seeds was recorded following four weeks of storage at 32 ^{o}C and 50 % relative humidity.

Following the storage of the seeds for four weeks in this study, there was a sharp decline in their germination capability with respect to all the treatment combinations. As reported by Hamim *et al*. (2014), the sharp decline in the quality of the seeds observed in this study is abnormal according to the normal and natural seed ageing process. The reason may be that the viruses **effects** must have been activated which resulted in the sudden and heavy decline in the germination percentages as reported by Ahmad *et al.* (2006). Furthermore, the variation in germination percentages amongst the cultivars and treatments as shown in this study suggest genetic superiority as reported by Anjorin and Mohammed (2014) and tolerance level of the cultivars over one another.

CONCLUSION AND RECOMMENDATIONS

The results of the experiment show that all the cultivars were susceptible to both single and mixed infections of the two viruses but to a seemingly different extent. The germination of seeds as seen from this study was generally high before storage but the high initial germination percentage was short lived. This is an indication that conservation of infected seeds of all the cultivars will be impaired. More so, all the cowpea cultivars did not exhibit dormancy which is a problem with most freshly harvested vegetable seeds. The benefits of increased cowpea production include improved nutrition for humans and livestock, improved soil properties and substantial opportunities for greater income.

The monitoring and management of these viruses is, therefore, crucial to sustainable cowpea production most especially in sub-Saharan Africa. There is, therefore, the need for constant monitoring of legume fields through regular disease surveillance, field sanitation as well as disease surveys to identify new and emerging viruses. This is because results from this work present a good starting point for legume virus diseases diagnosis in the study area. Finally, there is, also the need to ensure availability of acceptable horticultural desirable cowpea cultivars with high level of resistance to cowpea viruses for the nation to sustain its increased production.

Table 1: Cowpea seed quality as affected by single and mixed infections of *Blackeye cowpea mosaic virus* (BICMV) and *Cowpea mottle virus* (CPMoV) at Mokwa in 2017

	Germination Test (%)				Accelerated Ageing Germination (%) 4 Weeks of Storage			
Cultivar	BICMV	CPMoV	BI + CP	CP + BI	BICMV	CPMoV	BI + CP	CP + BI
Ife Brown	93.5^{bcd}	$90.5^{c\text{-}f}$	86.7^{f}	86.5^{gh}	77.9^{a}	69.0^{b}	56.6^{b}	46.2^{l}
IT90K – 277 – 2	94.7^{bc}	98.7^{a}	78.5^{c}	96.0^{bc}	76.6^{a}	68.0^{bc}	58.4^{a}	51.6^{e}
IT96D – 610	87.3^{g}	97.7^{a}	97.6^{b}	96.0^{bc}	70.3^{b}	66.5^{d}	52.0^{e}	52.0^{de}
IT97K – 499 – 35	88.0^{fg}	86.9^{i}	97.6^{b}	92.0^{e}	61.5^{de}	60.0^{g}	55.0^{c}	56.0^{ab}
IT97K – 568 – 18	99.7^{a}	91.2^{c}	81.3^{j}	98.5^{a}	69.5^{b}	57.2^{h}	48.0^{h}	41.2^{l}
IT97K – 573 – 2 – 1	87.8^{g}	93.4^{b}	94.8^{d}	94.5^{d}	50.6^{l}	57.1^{hi}	45.5^{i}	35.6^{m}
IT98K – 205 –M8	87.6^{g}	89.2^{efg}	77.5^{m}	96.0^{bc}	57.5^{gh}	70.6^{a}	56.3^{b}	41.5^{l}
IT98KD – 288	$91.3^{c\text{-}g}$	90.7^{cde}	82.6^{i}	96.0^{bc}	48.0^{m}	62.7^{f}	48.3^{gh}	51.3^{ef}
IT99K – 316 – 2	$92.1^{c\text{-}f}$	93.4^{b}	85.0^{gh}	97.3^{ab}	53.3^{jk}	64.0e^{f}	57.3^{ab}	46.0^{j}
IT99K – 377 – 1	88.9^{efg}	90.8^{cd}	85.4^{gh}	92.0^{e}	60.0ef	60.0^{g}	50.6^{f}	31.6^{n}
IT00K – 901 – 5	88.8^{efg}	86.1^{i}	81.3^{j}	89.3^{f}	70.6^{b}	65.0^{e}	52.0^{e}	47.0^{ij}
IT03K – 337 – 6	97.3^{ab}	$89.4^{d\text{-}g}$	84.6^{h}	89.3^{f}	50.5^{l}	66.8^{cd}	46.4^{i}	41.4^{l}
IT04K – 267 – 8	$92.2^{c\text{-}f}$	98.2^{a}	81.3^{j}	86.5^{gh}	56.0^{hi}	62.6^{f}	49.5^{fg}	54.6^{bc}
IT04K – 291 – 2	87.8^{g}	86.9^{i}	89.3^{e}	87.7^{g}	54.6^{ij}	58.7^{g}	57.4^{ab}	52.0^{de}
IT04K – 321 – 2	$90.5^{c\text{-}g}$	93.8^{b}	85.3^{gh}	78.6^{k}	58.6^{fg}	56.3^{hi}	48.0^{h}	50.6^{efg}
IT04K – 332 – 1	99.7^{a}	98.5^{a}	98.5^{a}	96.0^{bc}	60.0^{ef}	53.4^{k}	49.3^{fgh}	48.1^{hi}
IT06K – 124	93.7^{bc}	$90.1^{c\text{-}g}$	80.0^{k}	81.2^{j}	46.6^{m}	56.8^{hi}	58.6^{a}	49.6^{fgh}
IT06K – 137 – 1	77.4^{h}	87.2^{hi}	78.8^{l}	80.0^{jk}	52.0^{kl}	56.0^{hij}	54.5^{cde}	44.0^{k}
IT07K – 211 – 1 – 8	94.5^{bc}	88.5^{gh}	89.3^{e}	78.6^{k}	53.3^{jk}	56.0^{hij}	53.4^{d}	49.3^{gh}
IT07K – 222 – 2	92.5^{cde}	93.0^{b}	74.8^{n}	96.0^{bc}	54.2^{j}	56.4^{jk}	45.3^{ij}	53.7^{bc}
IT07K – 243 – 1 – 10	97.6^{ab}	98.4^{a}	89.3^{e}	94.8^{cd}	57.5^{gh}	54.7^{jk}	50.5^{f}	50.8^{efg}
IT07K – 251 – 3 – 3	92.3^{cde}	88.5^{gh}	82.8^{i}	85.3^{h}	57.3^{gh}	56.6^{ij}	44.0^{k}	47.0^{ij}
IT07K – 292 – 1 – 10	99.7^{a}	77.4^{j}	96.0^{c}	81.3^{j}	62.1^{d}	57.3^{h}	58.3^{a}	57.3^{a}
IT07K – 299 – 6	80.3^{h}	89.0^{gh}	86.8^{f}	82.6^{i}	64.4^{c}	64.0^{ef}	49.7^{f}	44.0^{k}
IT07K – 318 – 33	$89.3^{d\text{-}g}$	77.6^{j}	85.6^{g}	80.0^{jk}	59.8^{f}	58.7^{g}	44.9^{jk}	46.5^{ij}
SE ±	1.27	0.5	0.26	0.43	0.54	0.46	0.42	0.61

Means with the letter (s) within the same column are not significantly ($p \leq 0.05$) different by Student-Newman-Keuls (SNK) test

Comment [Anon2]: This is not explained anywhere

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