ASSESSMENT OF OKRA (*Abelmoschus esculentus*) CULTIVARS FOR SEED QUALITIES AND ISOLATION OF ASSOCIATED PATHOGENS IN NIGER STATE, NIGERIA

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Abstract

Assessment of the qualities of Okra cultivars and associated pathogens where investigated. Five okra cultivars NG-O-1, NG-O-2, NG-O-3, NG-O-4 and NG-O-5 were collected from five selected farms based on characterization and cultivation of the crop, soil types and topography, Niger State Agricultural and Development Programme divided the state into three agricultural zones namely Zone I, II and Zone III. Seed germination test, seed weight, size and seedling emergence were conducted in accordance to the method of International Rules for Seed Testing Association. Seed borne pathogens were isolated using agar plate methods. Results showed four fungal species associated were *Mucor pusillus*, *Aspergillus fumigatus*, *A. niger*, *Microsporum canis*, *Penicillium* species. Bacterial species isolated included *Bacillus subtilis*, *Micrococcus luteus*, *B. linchinoferus*, B. *megaterium* and *Pseudomonas aeruginosa*. NG-O-2 collected from Zone I recorded the highest germination rate (90%) while NG-O-1 and NG-O-3 from Zone II with the least germination rate (60%). Seed weight showed similar trend, with Zone I recording 5.85 g and NG-O-3 collected from Zone II had (0.47 mm) size which was the highest among the cultivars at ($P \geqslant 0.05$). Seedling emergence test showed that cultivars from Zone II had 86% while that from Zone I was 73%. Assessing okra cultivars and associated pathogens of the crop are important tool for improved for yield improvement in okra and will be useful in organic farming systems.

Keywords: Cultivars, diseases, okra, pathogens, qualities

Introduction

Okra (*Albemoschus esculentus*) is a flowering plant in the Mallow family. It is an annual or parental plant with origin from Africa and it is cultivated in the tropical, subtropical and warm temperate regions (Kazmi *et al*., 2013). The plant is an erect, coarse, robust annual herb in which flowers are borne finely in the leaf, axile on peduncles. It has a typical Malvaceous flora organization with eight to ten very narrow hairy, bracteoles forming an epicalyx. The fruits are long (10-30 cm); beaked, ridged, more or less oblong hairy capsules that dehiscing longitudinally. The seeds are enclosed in capsules which can split open when properly matured and dried. The leaves are about 10-20 cm long broad,

palmate lobed with 5-7 lobes and their flowers are about 4-8cm in diameter, with five white to yellow petals (Sorapong, 2013). Okra is known by many local names in different part of the world. It is called lady's finger in England, gumbo in United States of America (Gemede *et al*., 2015) guino-gombo in Spanish, guibeiro in Portuguese and bhindi in India. It is known as "Illa" in Yoruba, "Kubewa" in Hausa and "Okuru" in Igbo tribes of Nigeria (Gemah and Daagema, 2012). The importance of okra cannot be over emphasized. Okra contains carbohydrates, vitamins, proteins and vitamin C in large quantity (Ezerouye and Ubalua, 2005). The essential amino acids that okra has are comparable to soya-beans. Hence, it plays a vital role in human diet Adeghbehingbe (2014).

According to Umoru and Benjamin (2015), seed-borne disease refers to the particular plant diseases that are transmitted by seed. Seeds are the most important and cheapest input in crop production as 50 % gain in productivity is attributable to use of improved seeds.

The quality of seeds alone is known to account for an increase in productivity of at least 10–15 %. To achieve this high quality, all the factors in production that will affect seed viability, disease free and genetic purity should be taken into account. Bacterial species such as *Staphylococcus aureus, S. epidermidis, Micrococcus luteus, Pseudomonas aeruginosa,* and Proteus vulgaris they were found associated with okra samples. (Rahima and Dawar, 2015). Similarly, Kolawole, *et al.* (2011) reported that *Aspergillus* species, *Fusarium* species and *Macrophomina phaseolina* were found associated with seed- borne mycoflora of some okra leading to loss of the crop. Seed quality is the ability of seeds to germinate under a favourable environmental conditions and to develop into healthy seedlings (Kazmi, *et al.,* 2013). Simon *et al.,* (2013) reported that 83.3% of the farmers in Nigeria identified poor seed quality and planting materials as militating against cultivation of indigenous vegetables. They observed that seedling survival and mortality is a direct effect of poor seed quality and vigour. Crops with poor seed quality damages easily after sowing in field and during post- harvest as a result of pest and pathogen infestation (Nwangburuka *et al.,* 2013).

This study assessed seed qualities and seed borne microbes associated with some *Abelmoschus esculentus* cultivars from Niger State, Nigeria for possible guidance in selection in the emerging organic crop production systems..

Materials and Methods

Different seed cultivars of okra namely; NG-O-1, NG-O-2, NG-O-3, NG-O-4 and NG-O-5 were collected directly from selected farmer's field in Minna (NG-O-1), Zungeru (NG-O-2), Suleja (NG-O-3), Lapai (NG-O-4) and Kontagora (NG-O-5) areas of Niger State, Nigeria. Matured pods were collected from 52-day old okra plants and dried at room temperature for five (5) days and carefully hand threshed packed in paper bags and stored at ambient temperature *(28±2 °C)* in the Pathology Laboratory of Ibrahim Badamasi Babangida University, Lapai, Niger State.

Three replicates each of 100 seeds samples extracted were surface sterilized with 2% Sodium hypochloride (NaOCl) plated on Potato Dextrose Agar (PDA) and Nutrient Agar (NA) poured into 9 cm-diameter Petri dishes were incubated for 6-7

days at ambient temperature and arranged in accordance with International Rules for Seed Testing (I.S.T.A, 2010). Growth colonies of the isolates were repeatedly sub-cultured on Potato Dextrose Agar to obtain pure isolates of organisms. The identification of the isolated fungi was done by comparing them with confirmed representatives of different species using standard mycological identification key of Barnett and Hunter (1998). The morphological characteristics and microscopic observations of the bacterial and fungal species were carried out by adopting the methods of Atlas and Bartha (2007) and Cappucino and Sherman (2002).

Seed germination test

Hundred (100) seeds each from the samples were placed into moist sterilized filter papers contained in sterilized Petri-dish in triplicates and were kept moist for the period of ten days. The emergence of the plumule were observed and recorded daily. The germination percentage rate was calculated in accordance with I.S.T.A, (2010).

$$\text{Germination \%} = \frac{\text{Number of seed germinateed}}{\text{Number of seeds per Petri-dish}} \times 100$$

Seed weight and seedling emergence test

The weight of 100 seeds of each okra cultivars were determined and measured in grams using electrical balance (Model S-Metler, Model YP 502N England 2000). *Hundred (100) seeds of okra cultivars were sowed on sterilized topsoil on* 280 -200 - 80 mm plastic seedling trays at 20°C, under 12 hour of light and 12 hour of dark conditions. The test lasted for 22 days and at the end of the test, percentages of normal seedlings were determined by (I.S.T.A, 2010).

Determination of seed size

The diameter of 30 randomly selected seeds samples of each cultivar using the eye piece graticule, while the values of mean of seed length and width at the widest point were based on the measurement of 100 seeds samples of okra cultivars with the average recorded in milimetre (mm) Oluwajobi (2014).

Results

Results obtained showed that the seed mean weight was not significantly different ($P \leq 0.05$) between NG-O-4 (4.88g), NG-O-1 (4.77g) and NG-O-3 (4.46 g). Seed size were not significantly ($P \geq 0.05$) different in all the cultivars except in NG-O-3 which was largest (0.47 mm). Percentage emergence was not significantly ($P \geq 0.05$) different between NG-O-3, NG-O-4 and NG-O-5 with 86.6 % each (Table 1). There was also no significant different between NG-O-1 and NG-O-2 in mean % emergence.

A total five species of fungi from four genera (*Aspergillus fumigatus, A. niger, Microsporum canis, Mucor pusillus* and *Penecillium citrinium*) were isolated from the seeds and seedlings of the okra cultivars (Table 2). The percentage occurrence of *Mucor pusillus* was highest in the seeds from NG-O-1 (50 %), NG-O-4(75 %) and NG-O-5 (50 %). *Penecillium citrinium* was isolated in the seeds from NG-O-3 (25 %) while *A .niger* was isolated from only seedling from NG-O-3. *Aspergillus fumigatus* was isolated from NG-O-2 seedlings (50 %), NG-O-4 (25 %) and NG-O-5 (25 %) respectively.

Table I: Seed qualities of different Cultivars of Okra in Niger State

Cultivars	Seed Weight (g)	Size (mm)	Seed Germination (%)	Seedling Emergence (%)
NG-O-1	4.77±0.03^{a}	0.42±0.01^{a}	60.00±3.55^{a}	73.33±2.66^{a}
NG-O-2	5.85±0.07^{c}	0.44±0.01^{a}	90.00±5.77^{c}	73.33±2.67^{a}
NG-O-3	4.46±0.31^{a}	0.47±0.02^{b}	60.00±3.55^{a}	86.67±6.67^{b}
NG-O-4	4.88±0.12^{a}	0.43±0.01^{a}	73.33±6.67^{a}	86.67±6.67^{b}
NG-O-5	5.05±0.17^{b}	0.40±0.01^{a}	86.67±8.82^{b}	86.67±3.33^{b}

Means values ± S.E, Values followed by the same letter(s) along the column are not significantly different at p =0.05 as tested by Duncan Multiple Range Test.

Table 2: percentage occurrence of fungal species isolated from seeds and seedlings of Okra Cultivars

		Cultivars and percentage occurrence of fungal species				
	Fungal species	NG-O-1	NG-O-2	NG-O-3	NG-O-4	NG-O-5
Seeds	*Mucor pusillus*	50	0	0	75	50
	Penecillium citrinium	0	0	25	0	0
	Aspergillus fumigatus	0	25	25	0	25
	Microsporum canis	0	50	0	0	0
Seedlings	*Mucor pusillus*	75	25	25	25	50
	Aspergillus fumigatus	0	50	0	25	25
	Aspergillus niger	0	0	25	0	0

Five bacterial species were isolated from the seeds and seedlings of okra cultivars these include *Bacillus lichineforus*, *B. megaterium, B. subtilis, Micrococcus luteus* and *Pseudomonas aeraginosa* (Table 3). Percentage occurrence of all the isolates ranges between 25-50%, with *B. subtilis* been the highest with 50% in all the seedlings of the cultivars, however, there was absence of *Bacillus lichineforus, B. megaterium, Micrococcus luteus* and *Pseudomonas aeraginosa* in NG-O-1,NG-O-2,NG-O-3,NG-O-4 and NG-O-5 in the seeds and seedlings of these cultivars.

Table 3: Percentage occurrence of bacterial species isolated from seeds and seedlings of okra cultivars.

	Cultivars and percentage occurrence % of the bacterial species					
	Bacterial species	NG-O-1	NG-O-2	NG-O-3	NG-O-4	NG-O-5
Seeds	*Bacillus subtilis*	25	25	25	25	25
	Bacillus megaterium	25	25	0	0	0
	Micrococcus luteus	25	25	25	25	0
	Bacillus lichineforus	0	0	0	0	25
	Pseudomonas aeruginosa	0	0	25	0	0
Seedlings	*Bacillus subtilis*	50	50	50	50	50
	Bacillus lichineforus	25	0	0	25	25
	Micrococcus luteus	0	50	0	0	0
	Bacillus megaterium	0	0	25	0	0

Discussion

Seed weight recorded in NG-O-1, NG-O-2, NG-O-3, NG-O-4 and NG-O-5 (4.46-5.85mg) indicated that the okra cultivars might possess genetic impurity and this might lead to seedling blight disease. This was in line with the work of Sameh (2012) who reported on weight of okra seeds, that heavy seed weight values (4.00-6.00 mg) in okra seeds were less likely to emerge quickly when sowed in the soil thereby pre-disposing the seeds to soil-borne pathogens that causes seed rot and subsequent disease of okra seedlings. Similarly, the reports of Muhammed *et al.*, (2014) agreed with this study. The highest number of seed germination rate were NG-O-2 (90.0 %) NG-O-4 (73.3 %) and NG-O-5 (86.6 %), this revealed that the cultivars were of high quality. According to the reports of Chetty *et al.*, (2013) who reported 70-90 % germination rate in the evaluation of treated tomato seed for seed qualities are able to withstand seed and surface borne pathogens. This disagree with Anam *et al.*, (2002), who reported that 80% germination rate of untreated okra seed were one of the factors responsible for low seed yield. Similarly, Hassan (2002) recorded low yield in untreated rice and wheat. Seed size values of the okra landraces obtained in the study did not play any significant role in either germination or predisposing it to disease thus confirmed the report of (Moniruzzaman and Quamruzzaman, 2013).

In the present study *Bacillus subtillis, Bacillus megaterium, Bacillus lichineforus*, *Micrococcus luteus* and *Pseudomonas aeruginosa* were associated with the seeds and seedlings of the okra cultivars. This research corroborates with the

report by (Gemah and Daagema, 2012) who isolated some genera of *Bacillus* species, *Micrococcus luteus* and *Pseudomonas aeruginosa* from fresh okra samples. Kolawole *et al.* (2011) identified that some common microbial contaminants of Okra seeds and seedlings included *Staphylococcus aureus*, *Micrococcus canis* and *Bacillus* species leading to some seedling diseases in okra plants. Similar reports were obtained by Ezeronye and Ubalua (2005). Adeghbehingbe (2014) reported on microbial analysis of sundried okra samples from Akoko area of Ondo State Nigeria where *Bacillus subtilis, Staphylococcus aureus, Micrococcus cereus, Clostridium* species and *Pseudomonas aeruginosa* were isolated from the dried okra seeds. Frequency of occurrence fungal isolates was higher than that of bacterial isolates in seeds and seedlings (Table 2 and 3). *Mucor pusillus, Aspergillus fumigatus, Bacillus subtilis* and *Micrococcus luteus* percentage occurrence were found to be higher and most prevalent in all the different cultivars. *Aspergillus fumigatus*, *Aspergillus niger*, *Mucor pusillus, Microsporum canis* and *Penecillium citrinium* isolated from the seeds and seedlings of the okra cultivars revealed varying percentage occurrence from 25-75%. Two species of *Aspergillus* and *Mucor pusillus* are common in all the cultivars. This was earlier reported by Akter (2008) that *Aspergillus* species and some *Mucor* species were associated with okra seed mycoflora. Similarly, Youssef and Palmateer (2008) reported the isolation of *Aspergillus fumigatus*, *Aspergillus niger* and *Mucor pusillus* from stored Onion bulbs. Similarly, Muhammed and Muhammad (2013), also identified *Aspergillus niger, A. flavus* and *Penecillium* species from okra seedlings causing blight disease of okra in Niger State.

Okra plays a vital role in human nutrition by supplying the necessary growth factor such as vitamin, protein, minerals in the body (Adeboye and Oputa, 2006). Assessing okra seeds cultivars and their associated pathogens is very important so as not to predispose the seeds to infection when sown in gardens and fields as this could result to the reduction in the quantity and yields to farmers in Okra production.

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