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Original article

Evaluation of genetic integrity of tomato seeds during ageing by microsatellite markers

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Abstract

The objective of this project was to evaluate ageing-induced genetic changes during storage of tomato seeds. Seeds of 10 accessions of tomatoes were thus subjected to artificial ageing in chambers conditioned to 55 °C and 72 ± 2% RH for 72 h. Seed survival data were used to estimate probit parameters. Genetic changes during the seed ageing course were evaluated by microsatellite (SSR) analysis using a Direct PCR™ kit., The SSR primer sequences (except SGN-14430) optimized the gene markers for the tomato accessions and are thus recommended for detecting genetic changes during seed storage. Genetic distances were calculated using PAST™ software and percentage genetic integrity was estimated from the genetic distance matrices. Probit analysis showed that P_{50} estimates was least in accession LOO169 (7 days) and highest in accession 09/044 (64.4 days). Seeds having lower P_{50} estimates showed lower estimates of genetic integrity. Lowest estimate of average genetic integrity was 99.21% when estimates of genetic distances were compared between 0 and 72 h of artificial ageing. The results showed ageing-induced genetic deterioration during artificial ageing thus suggests possibility of losses in genetic integrity of seeds in storage at a specific physiological (germinability) benchmark. For tomatoes, this study shows that seed germinability benchmark for genetic changes during ageing was approximately 42%. Comparison of tomato seeds stored in the NACGRAB gene bank for 2 years and freshly harvested seeds, validated the artificial seed ageing result and the estimated germination benchmark for declining genetic integrity in tomato seeds.

Keywords: Seed longevity; Seed aging; Seed storage and genetic integrity

1. Introduction

Tomatoes are important fruit vegetables in Nigeria, the largest producer with 126,000 ha and an annual production of 879,000 tons [11]. Thus, there are concerted efforts by the National Center for Genetic Resources and Biotechnology (NACGRAB) to capture the genetic diversity for tomato improvement in Nigeria. Consequently, about 25 accessions of local landraces are maintained in a gene bank facility operating mostly at above zero temperatures. Under such conditions, maintenance of seed collections requires frequent seed sampling and regeneration. It is therefore logical to evaluate possible genetic drifts of seeds during the physiological ageing course in storage.

It is well known that ageing cause losses in physiological integrity even in prolonged gene bank storage under the best storage conditions, hence, seed viability models had been established and implemented for predicting seed longevity and management of gene bank seed collections of many crops including those of *Solanum* family (Daniel et al., 2011). Seeds

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of *Solanum* family were generally known to possess longevity ranging from intermediate lifespan to short-lived as reported for *Solanum macrocarpon* by Ref. [3]. Seeds of tomatoes are generally classified as short-lived and characterized by rapid decline in physiological integrity during storage at room conditions. This coupled with the harsh tropical production and storage temperature conditions and sub-standard gene bank storage necessitate the need to evaluate physiological changes during ageing of tomato seeds.

Genetic integrity is also of concern as much as the physiological integrity during seed storage. For example, genetic drifts during regeneration risks compromising the genetic integrity in form of changes in allele frequencies of populations over generations [7]; and for gene bank conserved seeds genetic integrity may reduce during regeneration due to mechanical, environmental and human errors [1]. Besides these, recent attempts to genetically analyze the seed longevity traits in artificially aged barley seeds had suggested that ageing can induce genetic drifts in seeds [8]. The objectives of this research were therefore to evaluate ageing-induced genetic changes for efficient management of seed gene bank collections and to derive physiological thresholds in terms of seed germination capacity for loss of genetic integrity during seed ageing in storage.

2. Materials and methods

Seeds of 10 accessions of the tomato collection at the NACGRAB gene bank in Ibadan, Nigeria were used in this trial. The seeds were multiplied in the field in 2013 and the harvested seeds were subjected to artificial ageing. Artificial ageing was done by placing 700 to 1000 seeds of each tomato accession in a chamber set at 55 °C and conditioned to 72 ± 2% RH using wet towels. Seeds were drawn for sampling at intervals of 3, 6, 12, 24, 36, 48 and 72 h for seed germination and DNA tests. Germination tests were conducted on 20 seeds using moist paper substrate for 8 days. The percentage germination data over time (survival data) were subjected to probit analysis using SAS™ to estimate seed longevity parameters for each accession. DNA analyses were conducted on the seeds with the Phire Plant Direct PCR™ kits, 50 to 100 Kb DNA ladder, GR-Green™ gel stain and Uvtech™ gel documentation system for gel visualization. Five highly polymorphic microsatellite (SSR) primers for tomatoes were sourced from the SOLGEN sequence database (Table 1), and the sequences were prepared by Inquaba Pty., South-Africa. The SSR primers and digested seeds were amplified with optimized PCR reactions. The PCR products were analysed by Poly-Acrylamide gel electrophoresis and the DNA profiles were analysed using the PAST™ software to generate genetic distances between 0 h and each of the other hours of ageing. Estimates of average percentage genetic integrity were derived from the genetic distances for each of the ageing hour and each tomato accession according to Ref. [4]:

$$\%\text{Genetic integrity} = 100 - \sum[(X_1 \& X_n)/n]$$

where: $X_1 \ldots .X_n$ = Values of genetic distances between seeds aged for 0 h and each of the ageing hours estimated by PAST™ software; n = number of observations.

To validate the results in the artificial ageing experiment, germination and DNA tests were conducted on fresh seeds of five selected accessions harvested in 2013 in comparison with seeds of the same accessions produced and stored in 2011 for 2 years at 2 ± 1 °C in the NACGRAB gene bank.

Table 2
Seed germination during artificial aging (survival) of 10 tomato accessions.

Accession	Seed germination[a]					
	0hr	3 hrs	24 hrs	48 hrs	55 hrs	72 hrs
NG/SA/01/10/002	67.21	58.95	58.95	50.77	52.24	48.85
NG/AA/SEP/09/050	70.09	63.44	46.43	60.00	48.85	53.73
LOO 170	53.73	38.30	40.22	36.27	38.29	27.76
LOO 169	52.77	21.47	25.40	16.85	29.00	21.47
NG/RM/JAN/10/001	54.76	60.00	47.87	44.08	40.22	27.76
NG/MR/MAY/09/006	67.21	62.31	55.24	50.37	58.37	52.77
NG/AA/SEP/09/037	71.56	61.14	65.27	55.80	53.73	46.43
NG/AA/SEP/09/044	73.26	68.61	58.37	58.37	52.24	53.73
NG/AA/SEP/09/053	75.11	60.00	56.79	55.80	46.95	41.21
LOO 166	71.56	53.73	52.77	55.80	45.00	43.11

[a] Arc sine values.

3. Results and discussion

Seeds of all tomato accessions exhibited declining germination capacity during the artificial ageing experiment (Table 2) shown by the negative values of the slope parameter from probit analysis of the seed survival data (Table 3). Physiological declines ultimately led to wide differences in P_{50} estimates among the 10 accessions, accession LOO169 showing extremely low values of $P_{50} = 7$ h while P_{50} values of seeds of other accessions ranged from 25 to 64.4 h. The range of P_{50} values shows that tomato seeds can be grouped into the long-lived seed type based on the comparative longevity testing protocol of Millennium

Table 1
Sequences of the SSR primers tested on the seed samples.

SSR primers	Forward sequence	Reverse sequence
SGN-E111025	AAGAAGAAGGATCGATCGAAGA	CATGACCACGATACTACATGTTT
SGN-E14430	GCAGCATATATCACCTTGGCT	CGTGCTCTCCAATAGTTCACC
SGN-M15396	GCATCATGAAATCACAATCAAAAA	CGGAAAAGAAATGAGACAAAGAA
SGN-TGS209	TGCAAAAATCGAAAATCGAA	GGGAAAATTTTATGTTAAGCTCCA
SGN-M15617	CGTACTCTCGTCCATCTTCATCA	GAATGCTTCAAAAAGTTGATTCG