



A novel genotypic selection methodology to create a breeding population for developing drought-tolerant cultivars in tree crops [☆]



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ARTICLE INFO

Method name:

Genotypic selection methodology to create a breeding population for developing drought-tolerant cultivars in tree crops

Keywords:

Coconut
Climate change
Environmental stress
Population improvement
Yield stability

ABSTRACT

With the adverse effects of climate change, selecting the best tolerant varieties to drought stress is highly necessary to sustain the yield and productivity of agricultural crops including tree crops. However, classical drought tolerance selection studies of tree crops have several limitations due to their relatively long lifespan. In this study, we propose a method to identify stable high-yielding trees under changing soil moisture conditions using yield data of existing elite tree populations. We develop this method using the data from a tropical tree palm, Coconut (*Cocos nucifera* L.) as a model crop. Our selection method considers individual palms as different genotypes. The method considered both mean trait values and their stability across different environments therefore, it can be effectively used to identify elite genotypes of tree crops for drought tolerance.

- We propose an analysis framework to identify stable, high-yielding individuals of tree crops under limited soil moisture conditions using the data of existing tree populations.
- Individual trees that produce stable and higher yields under soil moisture stress were identified based on mean yield and regression-based coefficient over different environments characterized by inter-annual rainfall variability.
- Individual trees selected by this method can be used as parental populations in breeding programs that focus on developing drought-tolerant varieties.

Specification table

Subject Area:	Plant Breeding
More specific subject area:	Coconut Breeding
Method name:	Genotypic selection methodology to create a breeding population for developing drought-tolerant cultivars in tree crops
Name and reference of the original method:	C.R.K. Samarasinghe, M.K. Meegahakumbura, D.P. Kumarathunge, H.D.M.A. C. Dissanayaka, P.R. Weerasinghe, L. Perera. Genotypic selection approach made successful advancement in developing drought tolerance in perennial tree crop coconut. <i>Scientia Horticulturae</i> 287 (2021) 110,220. https://doi.org/10.1016/j.scienta.2021.110220
Resource availability:	N.A

[☆] **Related research article** C.R.K. Samarasinghe, M.K. Meegahakumbura, D.P. Kumarathunge, H.D.M.A. C. Dissanayaka, P.R. Weerasinghe, L. Perera. Genotypic selection approach made successful advancement in developing drought tolerance in perennial tree crop coconut. *Scientia Horticulturae* 287 (2021) 110220. <https://doi.org/10.1016/j.scienta.2021.110220>

DOI of original article: [10.1016/j.scienta.2021.110220](https://doi.org/10.1016/j.scienta.2021.110220)

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<https://doi.org/10.1016/j.mex.2023.102217>

Received 22 January 2023; Accepted 9 May 2023

Available online 10 May 2023

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Introduction

Drought stress negatively affects crop growth and yield due to its adverse impacts on the physiological processes of crop plants, including photosynthesis, respiration, and dry matter partitioning [1,2]. In the future, it has been predicted that evapotranspiration will increase more than precipitation in many regions of the world [3]. Hence, with increasing temperatures, plant growth is likely to be more constrained by available soil moisture [4–6]. Compared to the annual and bi-annual crop species, the economic lifespan of tree crops is higher therefore, tree crop growth and yield are frequently negatively affected by the temporal variation in soil moisture under field conditions. Additionally, due to the longer lifespan, it is highly likely that a tree crop seedling established in the field today will face the projected drought changes in the future environment within the economic lifespan. Hence, selecting the best tolerant varieties to drought stress is highly necessary for tree crop yield and productivity in future relatively dry environmental conditions. Thus, breeding crop varieties for drought tolerance has become a high priority research theme at present for most of the agricultural crops including tree crops.

Breeding tree crops for drought tolerance is challenging due to several reasons. Firstly, most of the tree crops take 3 to 6 years to reach the reproductive stage, therefore the time taken to complete a breeding program through classical methods (ex. hybridization followed by progeny selection) is relatively higher compared to an annual crop species. Secondly, the high degree of heterozygosity, lack of marker availability, financial limitations etc. also restricts the applicability of modern breeding techniques such as Marker Assisted Selection for tree crop breeding [7,8]. However, genotypic improvement of the population by incorporating favorable genes through intermitting best drought-tolerant and high-yielding trees is one of the practical methods used in breeding for drought tolerance in tree crops.

A population improvement program for drought tolerance of tree crops should necessarily hasten the selection process for stress tolerance as well as for high yield. Classical drought tolerance selection studies of tree crops (ex. Coconut) are usually conducted under the framework of multilocal environmental trials where cultivars are grown in replicated trials in locations with contrasting rainfall patterns [9,10]. However, conducting multilocal trials with tree crops with relatively long lifespan is difficult due to the requirement of maintaining the experiment at multi locations for several years (10–15 years or more) as tree growth can be hampered by other factors such as biotic stresses. Therefore, having a relatively cost-effective and easy-to-practice selection procedure is of paramount importance in breeding tree crops for drought tolerance.

In this paper, we propose a method to identify stable high-yielding trees under limited soil moisture conditions using the data of an existing elite tree populations as the base population. Using existing elite populations which are already cultivated is a good source for the use as the base population because they were already evaluated for other traits such as yield quality parameters. We develop this method using data from a tropical tree palm, Coconut (*Cocos nucifera* L.) as a model tree crop. We explicitly select coconut for this study as the economic lifespan of coconut is about 60–65 years, therefore, a tree established in the field experience a wide array of rainfall changes. Therefore, it is highly necessary to select the best tolerant varieties to environmental stresses in order to sustain the coconut yield and productivity in relatively dry environmental conditions. Our objective is to identify trees which produce stable and higher yields under varied climatic conditions over several years from a single site.

Method details

Theoretical background

In coconut, the entire developmental process from floral initiation to fruit maturity takes over two years [11]. It shows an indeterminate growth pattern and produces an inflorescence at intervals varying from 25 to 30 days, under favorable soil moisture conditions [12,13]. A healthy coconut palm contains 12–14 inflorescences (bunches) with varying numbers of fruits at different developing stages. Environmental variation experienced by the palm throughout this entire development process could affect the fruit set and fruit development, final nut yield and the nut quality of coconut [14]. However, moisture stress during the time of floral primordia initiation and temperature stress at the time of nut setting (i.e. 3 to 4 months after flower opening) are considered most critical for yield determination [13,14]. Given the relatively longer time taken to floral initiation to harvest, it can be assumed that the coconut bunches harvested at a given time (generally in two-month interval, 6 harvests per year) subjected to different environment stress during its development period. Hence, for coconut palms growing in regions where there is a considerable inter-annual variation in rainfall, each year can be considered as a different environment with respect to soil moisture availability. Therefore, annual yield data of individual palms recorded over several years can be considered as yield data records of individual genotypes (i.e. palms) at different environments with different soil moisture levels. Hence, these data can be effectively used to screen palms that produce stable yields within a range of soil moisture levels and those palms can be considered as tolerant to drought stress [15].

Method

Our selection method considers individual palms as different genotypes. Tall coconuts which are extensively cultivated as plantations across the globe are highly cross pollinating and the molecular methods also support using each palm as different genotype [16]. We obtained six years of annual nut yield records of 220 coconut palms (from 29 to 35 years old), belonging to two improved tall coconut cultivars (CRIC 60 and Ambakelle Special) in Isolated Seed garden (ISG), which is located in Dry zone of Sri Lanka. The ISG Ambakelle managed under rain fed condition without irrigation and regularly this site subjected to two distinct dry spell within each years. In this study, the regression based approach was used to select individual palms across that produce stable yield over

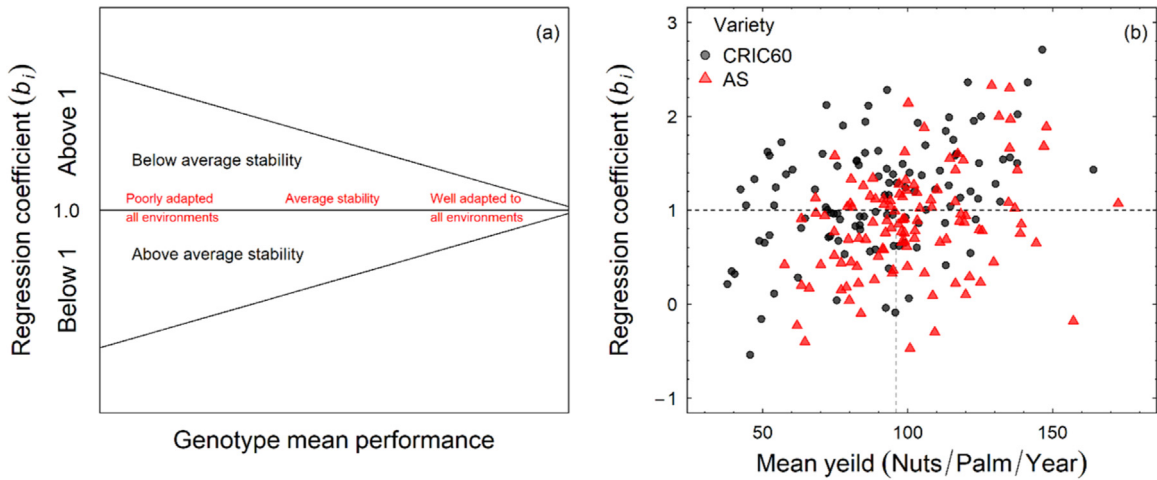


Fig. 1. Genotype regression coefficient against genotype mean performance (Finlay and Wilkinson, 1963) used for categorize genotypes based on adaptability to the environment (a). Regression coefficient (b_i) against mean performance for each palm in two variety groups (b) (This Figure was published in Scientia Horticulturae 287 [10220] Samarasinghe et al. Genotypic selection approach made successful advancement in developing drought tolerance in perennial tree crop coconut. pp:04 Copyright Elsevier [2021]).

	A	B	C	D	E	F	G	H
1	Genotype	Environment 1	Environment 2	Environment 3	Environment 4	Environment 5	Environment 6	
2	4677	127	59	86	27	70	67	
3	4708	115	59	78	56	15	25	
4	4720	145	117	127	64	10	56	
5	4722	121	122	136	81	26	53	
6	4724	84	13	151	113	88	126	
7	4726	148	159	160	57	59	118	
8	4728	133	110	125	83	42	111	
9	4732	213	205	155	141	57	78	
10	4808	83	81	33	29	35	43	
11	4828	141	98	93	138	41	67	
12	4830	114	108	84	75	12	101	
13	4832	131	44	93	66	52	115	
14	4834	43	122	144	142	68	84	
15	4836	89	137	109	93	60	106	
16	4838	173	224	202	110	22	148	
17	4840	131	54	13	16	20	93	
18	4842	130	113	122	104	39	121	
19	4844	128	98	68	69	13	48	
20	4845	29	14	79	37	23	45	
21	4846	150	36	67	32	12	42	
22	4849	29	66	62	42	33	92	
23	4850	34	37	53	54	46	73	
24	4911	92	105	106	35	42	75	
25	4915	141	42	104	78	25	109	
26	4919	132	107	108	113	33	64	
27	4925	139	100	112	87	49	75	
28	4931	122	96	110	117	87	147	
29	4935	182	114	104	77	32	177	
30	4937	105	96	39	65	29	113	
31	4939	61	93	79	47	20	80	
32	4940	121	55	99	58	21	8	
33	4941	61	94	61	58	22	18	

Fig. 2. A sample data input file for STABILITYSOFT. Variables should be entered in a spreadsheet as a Matrix of trait values of each genotype in each environment.

varying soil moisture conditions. This method was originally introduced by Finlay and Wilkinson [17] for analyzing genotype × environment interaction in multilocal environmental trials. In this method, a regression coefficient (b_i) is defined as the response of the genotype to the environmental that is derived from the average performance of all genotypes in each environment. The $b_i > 1$ indicates genotypes with higher sensitivity to environmental change and greater specificity of adaptability to high yielding environments, whereas a $b_i < 1$ describes a measure of greater resistance to environmental change, thereby increasing the specificity of adaptability to stressful environments, here in our case, years with limited soil moisture. If b_i does not significantly differ from 1, then the genotype is adapted to a wide range of environments. [17]. A conceptual depiction of the method is given in Fig. 1a. and the regression coefficient (b_i) is calculated using Eq. (1) [17,18].

$$b_i = 1 + \frac{\sum_i (X_{ij} - \bar{X}_i - \bar{X}_j + \bar{X}_{..})(\bar{X}_j - \bar{X}_{..})}{\sum_i (\bar{X}_j - \bar{X}_{..})^2} \tag{1}$$

$$s_{di}^2 = \frac{1}{E-2} \left[\sum_i (X_{ij} - \bar{X}_i - \bar{X}_j + \bar{X}_{..})^2 - (b_i - 1)^2 \sum_i (\bar{X}_j + \bar{X}_{..})^2 \right] \tag{2}$$

where X_{ij} is the trait value (i.e. nut yield) of the i^{th} genotype in the j^{th} environment, \bar{X}_i is the mean trait value of the i^{th} genotype, and \bar{X}_j is the mean trait value of the j^{th} environment, $\bar{X}_{..}$ is the overall trait mean and E is the number of environments. For example,

	A	B	C	D	E
1	Genotype	Y	s²d_i	b_i	
2	7352	45.67	147.75	-0.54	
3	6950	100.83	1415.44	-0.47	
4	6810	64.50	537.52	-0.40	
5	7104	109.33	391.97	-0.30	
6	6892	61.83	398.50	-0.23	
7	6867	157.17	603.59	-0.18	
8	4850	49.50	132.63	-0.16	
9	6935	83.83	95.39	-0.10	
10	4724	95.83	1612.80	-0.09	
11	7348	92.50	891.74	-0.04	
12	6859	79.83	68.39	0.04	
13	7318	75.50	909.24	0.04	
14	4834	100.50	1243.18	0.06	
15	6862	108.67	1058.55	0.09	
16	6895	120.00	478.61	0.10	
17	4849	54.00	403.93	0.11	
18	6947	77.00	299.49	0.15	
19	6773	66.00	106.66	0.17	
20	7020	78.83	209.81	0.18	
21	6902	63.33	256.08	0.20	
22	4845	37.83	356.28	0.21	
23	6865	116.50	133.67	0.22	
24	6976	83.00	44.80	0.22	
25	6937	125.17	67.79	0.23	

Fig. 3. A sample output file of STABILITYSOFT which show the trait values of each genotype (Y) and stability parameters S²d_i and b_i. A detailed output can be found as supporting information for this article.

here we have recorded nut yield of 220 genotypes in six different environments (years with contrasting rainfall) therefore, i ranged from 1 to 220 and j ranged from 1 to 6.

The regression-based method screens only the genotypes, i.e. palms in our case, which produce relatively stable yields across a wide array of soil moisture environments. However, this method does not provide a selection method for higher yields. Hence, we recommend selecting the palms which are having both b_i values less than 1 and a mean yield greater than a predetermined value. We recommend using individual palms which are having a mean annual nut yield greater than the grand annual mean yield + 2 standard deviations. After obtaining both the mean yield and b_i value of each genotype, plotting the regression coefficient (b_i) against the mean performance of each palm was evaluated to identify stable higher yielded palms (Fig. 1b) which are having above-average stability ($b_i < 1$) and higher genotype mean performance (genotype mean yield > Site mean yield).

The palms selected by the regression coefficient were further screened for stable yields using another regression-based approach called deviation from the regression (S^2d_i) proposed by Eberhart and Russell [19] (Eq. (2)). The variance of deviations from the regression (S^2di) introduced by Eberhart and Russell [19] is also used for the selection of stable genotypes. Genotypes with an $S^2di = 0$ would be the most stable, while an $S^2di > 0$ would indicate is lower stability across all environments. Hence, genotypes with lower values are identified as the most desirable [19]. The formula used to calculate the variance of deviations from the regression ($S^2 di$) [19]. We calculated S^2d_i values Eq. (2) for each palm and used those values as the second selection criteria. The palms with higher S^2d_i values are rejected from previously selected palms based on the b_i and mean yield. Traits such as nut quality parameters viz. kernel weight, oil content can introduce as final selection criteria and trees that produced a mean trait value lower than the population mean trait value should be rejected.

We recommend using the STABILITYSOFT software Pour-Aboughadareh et al. [20] to calculate the stability parameters S^2d_i and b_i . The software is available online (<https://manzik.com/stabilitysoft>) and the step-by-step guide for the calculations can be found on the software homepage. Briefly, the user must upload datafiles in spreadsheet format and the software output provides a range of variance components and stability parameters for the traits of interest. A sample data input file and output file are given in Figs. 2 and 3 respectively.

Our method considers individual years with contrasting rainfall as different environments, therefore providing new insight into analyzing genotype \times environment interaction without conducting multilocal environmental trials. Hence, our method provides a relatively cost-effective and easy-to-practice selection procedure for screening drought-tolerant individuals for population improvement programmes. Additionally, the method considers three aspects of yield; quantity, quality and stability therefore, the selected individuals can be considered as the best set of individuals in terms of environmental stress tolerance. However, the success of the method depends on the inter and intra-annual variation of rainfall (or the climatic parameter of interest). Therefore, the method can only be applied in environments where there is a significant variation in climatic variables of interest within and between years. The accuracy of the proposed method can be further increased by evaluating the progenies of the selected parents in multilocal environmental trials and subsequent selection.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgement

The authors acknowledge the past and present staff members of the Genetics and Plant Breeding Division of the Coconut Research Institute who supported in planting and maintaining the research blocks at Ambakelle Seed Garden. The authors also wish to thank the NRC research grant (18–084) for supporting this research project.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.mex.2023.102217](https://doi.org/10.1016/j.mex.2023.102217).

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