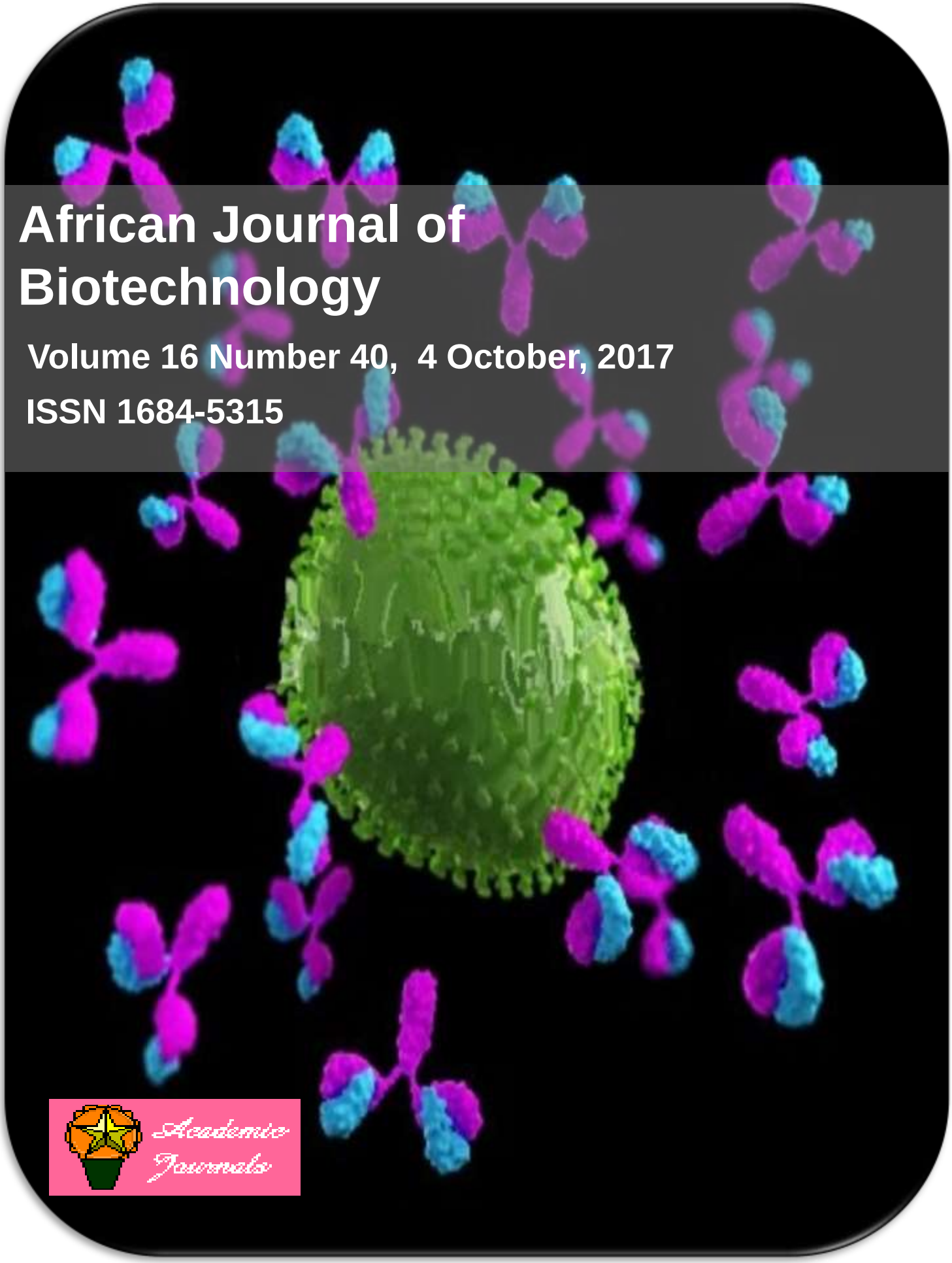

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Full Length Research Paper

Adsorption of a cationic dye by Marula (*Sclerocarya birrea)* fruit seed shell based biosorbent: Equilibrium and kinetic studies

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Adsorption of methylene blue from aqueous solution using a low cost *Sclerocarya birrea* fruit shell powder based biosorbent was investigated in a batch system. Results showed that optimum adsorption capacity was achieved at pH 8 and biosorbent dosage of 0.6 g, with a maximum adsorption capacity of 27.690 mg g^{-1}. Equilibrium studies showed that experimental data fitted well on the Temkin isotherm (R^2 = 0.9641), as well as the Langmuir isotherm (R^2 = 0.9626). The adsorption process followed the pseudo-second order rate kinetics with R^2 values greater than 0.999. Fourier transform infrared spectroscopy (FTIR) spectrum showed the presence of absorption bands typical of a plant based biomaterial. Given the abundant availability of the *S. birrea* trees in Southern Africa, the seed fruit shell can be used as a source of low cost biosorbent.

Key words: Biosorption, Langmuir, methylene blue, *Sclerocarya birrea* fruit, Temkin.

INTRODUCTION

Industrialization and urbanization, despite having contributed to improved living standards, have resulted in high levels of pollution in water bodies. Effluent dyes have been identified as one of the major sources of pollution in municipal waters. Wastewater from pulp and paper, tannery, textile, food, pharmaceutical and electroplating industries contains high levels of synthetic dye pollutants. These high levels of dye pollutants must be reduced below regulatory limits. Literatures suggest that there are more than 10,000 different types of synthetic dyes on the world market, with a combined annual production of 7 × 10^5 tons (Mohammed et al., 2014; Chen et al., 2003; Daneshvar et al., 2007; Aksu and Karabayır, 2008; Hameed et al., 2008). The manufacture of dyes and its application in the textile industries generate large volumes of effluent dyes, and this presents a great challenge in the treatment of such wastewaters (Joshi et al., 2004; Anjaneyulu et al., 2005;

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Kushwaha et al., 2014).

Synthetic dyes as water pollutants are a serious threat not only to human health but also to aquatic life. Health risks such as cancer development, mental confusion, tissue necrosis and a host of physiological disorders have been linked to exposure to high levels of synthetic dyes (Mathur et al., 2005; Puvaneswari et al., 2006). Effluent synthetic dyes also affect light penetration which is not conducive for aquatic life.

Despite the existence of a number of water effluent treatment technologies, challenges still exist. Some of these challenges include incomplete removal of dye and generation of secondary pollution (Babu et al., 2007). These established technologies include various biological and chemical treatment methods, adsorption, reverse osmosis and membrane filtration methods (Ong et al., 2011). One of the ways of controlling pollution is the introduction of a number of policies by water authorities in both developed and developing countries. There are, despite the benefits, challenges of monitoring and implementation especially in developing countries, mainly due to associated costs (Wang et al., 2008; Blackman, 2010).

Of the adsorption techniques that are being continuously developed, biosorption has attracted a lot of research interests due to its renewable and low cost nature (Choudhary et al., 2015; Hameed and Ahmad, 2009). This study reports a biosorption approach for the removal of methylene blue from water effluent using a renewable natural adsorbent, *Sclerocarya birrea* fruit seed shell powder. *S. birrea* is a naturally growing tree in Southern Africa (Neo et al., 2011). The fruits have a number of commercial uses and have been used to sustain livelihoods of indigenous population in Southern Africa (Wynberg et al., 2003; Jama et al., 2008). The shell is a by-product of fruit processing or consumption.

MATERIALS AND METHODS

Preparation of adsorbent

S. birrea fruit seed shells were harvested from a forest in the Chibi District of Masvingo Province in South Western Zimbabwe. The shells were washed with distilled water before being dried at 50°C overnight. The dried seed shells were ground into a fine particulate powder and were sieved through a 75-micron sieve. The powder was used for adsorption experiments without further treatments. The FTIR spectra of the powdered fruit seeds shell was recorded on a Thermo Fisher Scientific Nicolet iS5 MIR FTIR spectrophotometer equipped with an attenuated total reflectance (ATR) accessory and OMNIC software.

Adsorption experiments

Methylene blue solutions of 20, 40, 60 and 80 mg L^{-1} were prepared by serial dilutions from a 1000 mg·L^{-1} stock solution. In a 50 ml solution, 0.6 g of biosorbent was suspended and agitated at 200 rpm under pre-determined conditions. The concentration before and after adsorption experiment were determined using a Thermo Fisher Scientific Genesy 10S UV/Vis spectrophotometer measured at 664 nm. Adsorption parameters were optimized in terms of contact time, pH, and biosorbent dosage. Removal efficiency *(RE)* and adsorption capacity at equilibrium was calculated using Equations 1 to 3, respectively.

$$RE = \frac{C_0 - C_t}{C_0} \times 100 \quad (1)$$

$$q_t = \frac{(C_0 - C_t)V}{1000 \times M} \quad (2)$$

$$q_e = \frac{(C_0 - C_e)V}{1000 \times M} \quad (3)$$

Where, C_0 is the initial dye concentration in mg L^{-1}, C_t is dye concentration at time *t*, q_t is the adsorption capacity at time *t,* q_e is the adsorption capacity at equilibrium, Ce is the concentration at equilibrium, *V* the volume of dye solution and M the mass of biosorbent.

RESULTS AND DISCUSSION

FTIR spectrum

The FTIR spectrum of biosorbent powder is shown in Figure 1. A broad absorption band with peak maxima at 3334 cm^{-1} can be attributed to -OH stretching vibration. The band may also overlap -NH stretching vibrations and the amino group. A small but sharp absorption band at 1735 cm^{-1} can be attributed to the carbonyl group while strong absorption band is associated with a C-O-C stretching vibration. These IR absorption bands are typical of a number of plant biomaterials (Pavan et al., 2008; Song et al., 2011; Samiey and Ashoori, 2012; Hassan et al., 2017).

Effect of pH

The effect of sorbate solution pH on biosorption capacity was determined within the pH range of 2 to 10. The results are illustrated in Figure 2. It can be observed from the diagram that the biosorption capacity steadily increased to maximum adsorption capacity of 37.001 mg g^{-1} at pH 8 and fell drastically thereafter. A decrease in biosorption capacity below pH 8 can be attributed to increase in protonation of adsorption sites resulting in the repelling of diprotonated methylene blue molecules. A similar trend has also been observed for the biosorption of methylene blue by meranti sawdust based biosorbent (Ertugay and Malkoc, 2014; Ahmad et al., 2009).

Effect of contact time

Contact time parameter is an important parameter in the optimization of an adsorption process. The effect of

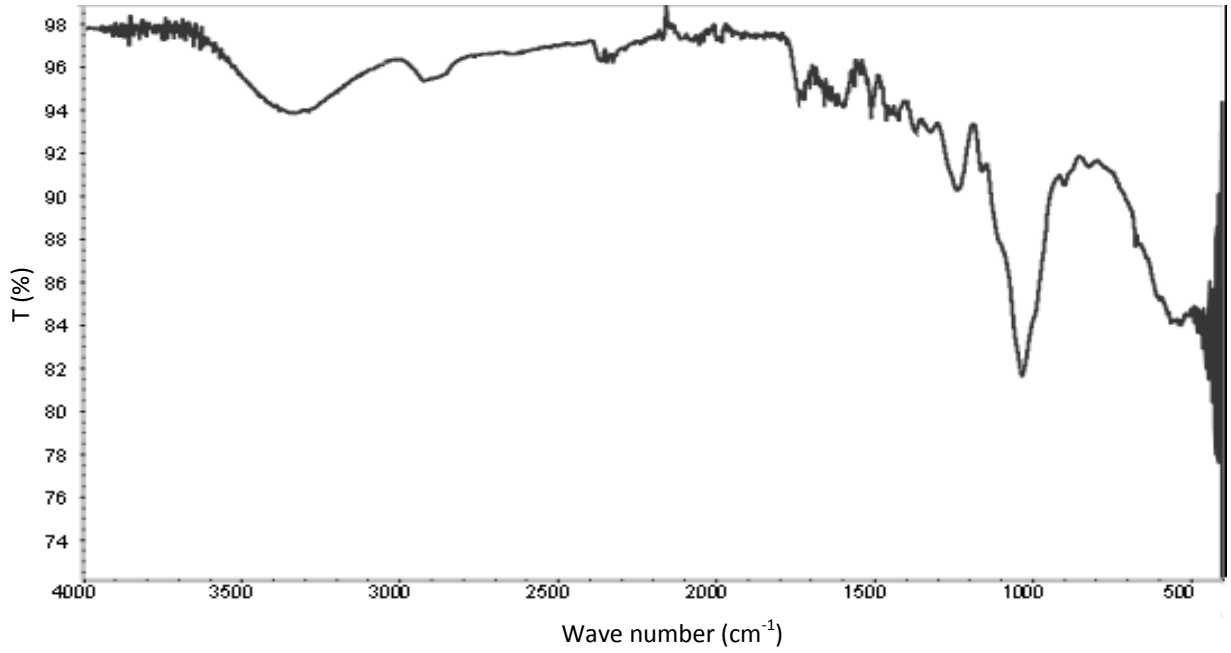

Figure 1. FTIR spectrum of *Sclerocarya birrea* fruit shell powder.

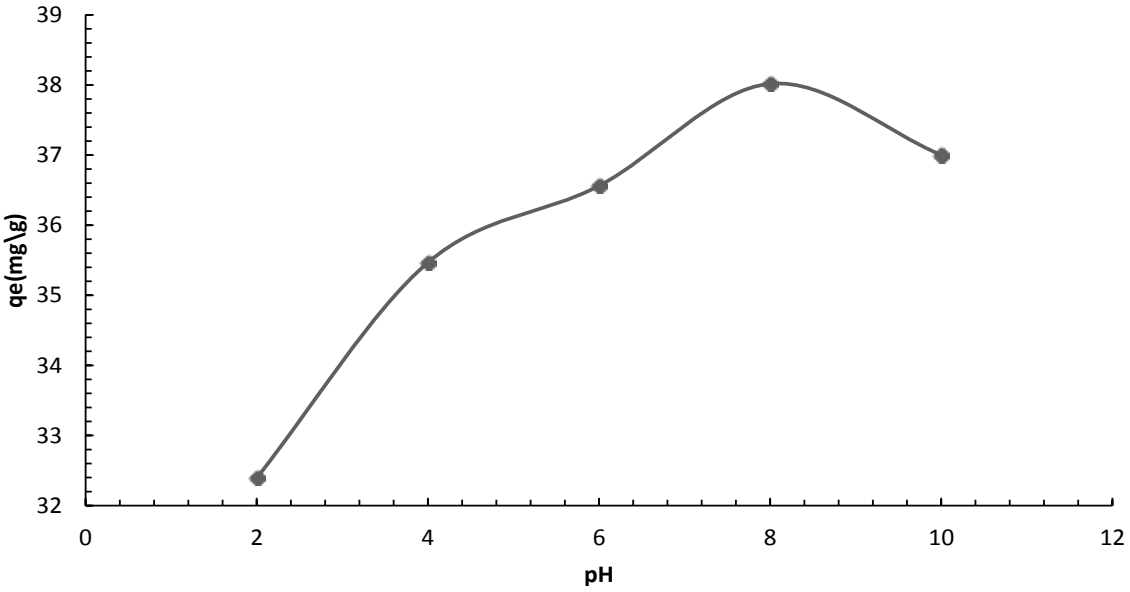

Figure 2. Effect of pH on the adsorption capacity of biosorbent of MB (C_0 = 80 mg·L^{-1}, biosorbent dosage = 0.6 g, agitation = 200 rpm).

contact time for initial concentrations 20, 40, 60 and 80 mg L^{-1} was monitored over 90 min and the result is illustrated in Figure 3. The diagram shows that for all initial concentrations, equilibrium was quickly reached in less than 10 min and this is mainly due to available adsorption sites. A biosorption process where equilibrium is quickly reached has also been observed using a brown alga based biosorbent (Caparkaya and Cavas, 2008).

Effect of biosorbent dosage

The effect of biosorbent dosage was determined for the dosage range of 0.1 to 1.0 g suspended in 50 ml of dye solution. The result is illustrated in Figure 4. From the figure, it can be observed that removal efficiency increased up to a dosage of 0.5 g and no increase was observed at higher dosages. The observed trend has

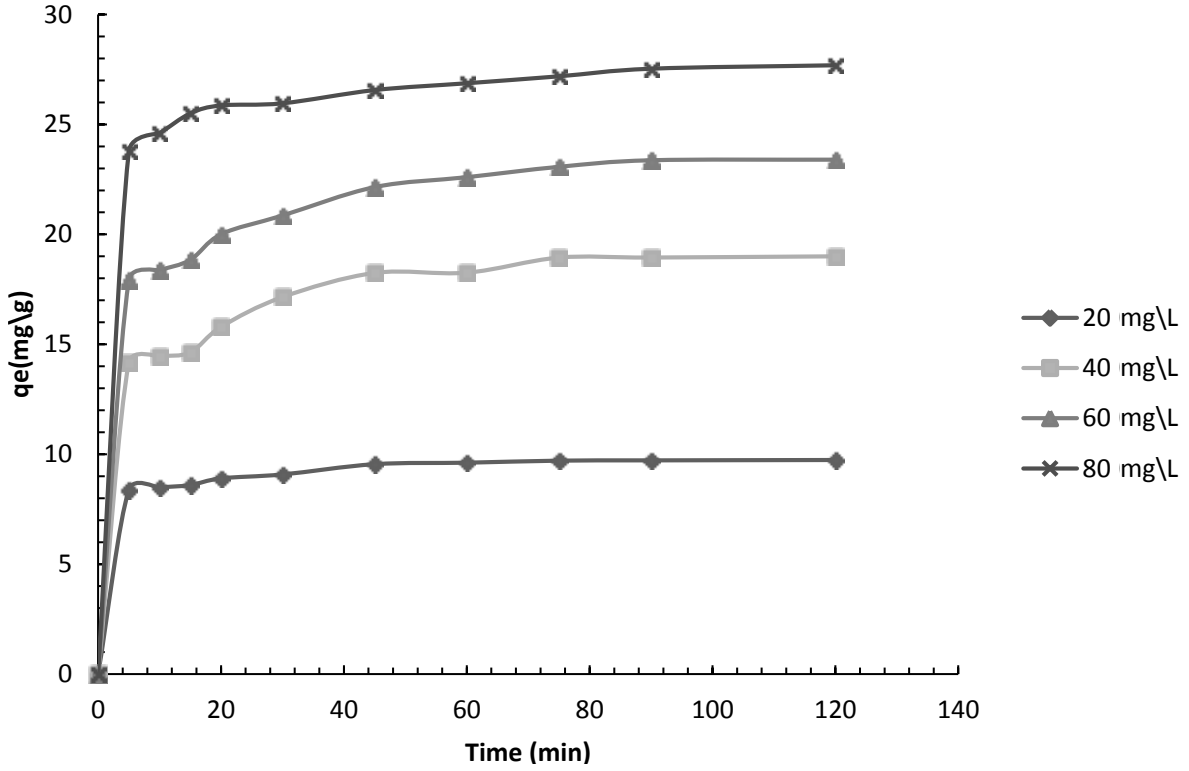

Figure 3. Effect of contact time of biosorption of methylene blue at different initial concentrations (pH = 8, agitation = 200 rpm, biosorbent dosage = 0.6 g, T = 298K).

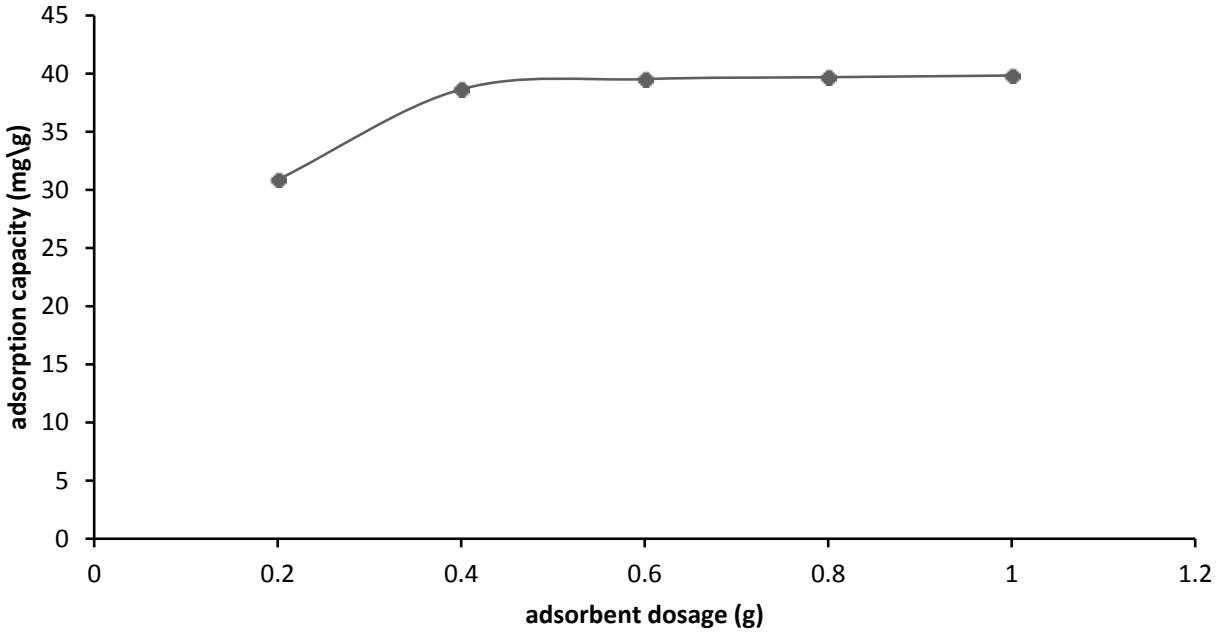

Figure 4. Illustration of the effect of biosorbent dosage of MB biosorption by *S. birrea* (pH = 8, C_0 = 80 mg·L^{-1}, agitation = 200 rpm, T = 298 K).

been attributed to the fact that at higher dosage, the effective surface area available for biosorption decreased as a result of aggregation (Guo et al., 2014; Barka et al., 2011).

Adsorption isotherm studies

Many empirical models have over the years been developed, but in this study, experimental data was fitted onto the Langmuir, Freundlich, Temkin and Dubinin-Radushkevick isotherms. The Langmuir adsorption isotherm model, the most common of all the models, assumes that the adsorbent has finite sites per unit mass that can be occupied by adsorbate molecules. The model assumes a monolayer adsorption on a homogeneous surface and that there is a finite number of identical sites (Langmuir, 1916). An expression of Langmuir adsorption isotherm is shown in Equation 4.

$$q_e = \frac{q_m K_L C_e}{1+K_L C_e} \quad (4)$$

Where, q_m is the maximum adsorption capacity in mg g^{-1}, q_e is the adsorption capacity at equilibrium and K_L is the Langmuir constant in L mg^{-1}.

The Freundlich adsorption isotherm assumes a multi-layer adsorption on a heterogeneous surface. The isotherm is an exponential equation expressed as shown in Equation 5.

$$q_e = K_F C_e^{1/n} \quad (5)$$

Where, K_F is the Freundlich constant in $mg^{1-1/n}L^{1/n}g^{-1}$ and is an indication of relative adsorption capacity of the adsorbent while *n* is a constant indicating the intensity of adsorption.

The Temkin isotherm was developed to take into account the effect of indirect adsorbent-adsorbate interactions on adsorption (Temkin, 1941). It postulates that the heat of adsorption of the layer would decrease linearly with coverage due to these interactions. The Temkin isotherm is expressed as shown in Equation 6.

$$q_e = \frac{RT}{B} lnK_T C_e \quad (6)$$

Where, R is the gas constant, K_T (L mg^{-1}) and B (J mol^{-1}) are Temkin constants.

The Dubinin-Radushkevick adsorption isotherm is normally applied to express the adsorption mechanism with a Gaussian energy distribution onto a porous heterogeneous surface. An expression of the isotherm is shown in Equation 7.

$$q_e = q_s e^{-K_{ad}\varepsilon^2} \quad (7)$$

Where, q_s is the theoretical isotherm saturation capacity in mg g^{-1}, K_{ad} is a constant related to the free energy of adsorption per mole adsorbate (mol^2 J^{-2}) and ε is the Polany potential which is related to the equilibrium concentration as shown in Equation 8.

$$\varepsilon = RTln\left(1+\frac{1}{C_e}\right) \quad (8)$$

The linearized forms and plots are summarized in Table 1. The results for fitting experimental data onto the isotherm models are summarized in Table 2. A comparison of the R^2 values suggests that the data fitted well onto the Temkin isotherm, as well as the Langmuir isotherm. A biosorption study with *Xanthoceras sorbifolia* seed coat based biosorbent also produced a similar result (Yao et al., 2009).

Kinetic study

Kinetic parameters are important in optimizing an adsorption process. Experimental data for methylene blue biosorption by *S. birrea* was fitted on the pseudo-first and pseudo-second order kinetic models. The pseudo-first order kinetic model is represented by Equation 9.

$$\frac{dq}{dt} = k_1(q_e - q_t) \quad (9)$$

Where, k_1 is the pseudo-first order kinetic constant. The integrated form of this equation is represented by Equation 10.

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (10)$$

The pseudo-second order kinetic model can be expressed as shown in Equation 11.

$$\frac{dq}{dt} = k_2(q_e - q_t)^2 \quad (11)$$

Where, k_2 is the pseudo-second order kinetic constant. The linearized integrated form of Equation 11 is as shown in Equation 12.

$$\frac{1}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_t} t \quad (12)$$

The parameters k_2 and q_e can be obtained from the intercept and the slope of $\frac{t}{q_t}$ versus t, respectively. The results for pseudo-first and pseudo-second order plots are illustrated in Figures 5 and 6, respectively. A summary of pseudo-first order and pseudo second order parameters is shown in Table 3. From the R^2 values, it can be concluded that the biosorption process followed more the pseudo-second order kinetic rate than the pseudo-first order rate. A number of studies on biosorption of methylene blue with different biosorbents suggest that adsorption kinetics tend to be more of pseudo-second order rate than pseudo-first order rate (Mitrogiannis et al., 2015; El Sikaily et al., 2006; Hamdaoui and Chiha, 2007; Ahmad et al., 2009).

Table 1. Linearized and plots of adsorption models used in the study.

Isotherm	Equation	Linearized form	Plot
Langmuir	$q_e = \frac{q_m b C_e}{1 + bC_e}$	$\frac{1}{q_e} = \frac{1}{K_L q_m}\frac{1}{C_e} + \frac{1}{q_m}$	$\frac{1}{q_e}\ vs\ \frac{1}{C_e}$
Freundlich	$q_e = K_F C_e^{1/n}$	$\ln q_e = \ln K_F + \frac{1}{n}\ln C_e$	$ln\ q_e\ vs \ln C_e$
Temkin	$q_e = \frac{RT}{B} lnK_T C_e$	$q_e = \frac{RT}{B}\ln K_T + \frac{RT}{B}\ln C_e$	$q_e\ vs\ \ln C_e$
Dubinin-Radushkevich	$q_e = q_s e^{-K_{ad}\varepsilon^2}$	$\ln q_e = \ln q_s - K_{ad}\varepsilon^2$	$\ln q_e\ vs\ \varepsilon^2$

Table 2. Comparison of the adsorption isotherms parameter.

Langmuir isotherm	
q_m	27.690 mg·L^{-1}
K_L	0.0401 L·mg^{-1}
R^2	0.9626
Freundlich isotherm	
n	0.3017
K_F	2342.07 $mg^{1-1/n}L^{1/n}g^{-1}$
R^2	0.9270
Temkin isotherm	
B	2.1702 J·mol^{-1}
K_T	0.2074 L·mg^{-1}
R^2	0.9642
Dubinin-Radushkevick isotherm	
K_{DR}	23.007
ε	0.1406 mol^2·J^{-2}
R^2	0.9382

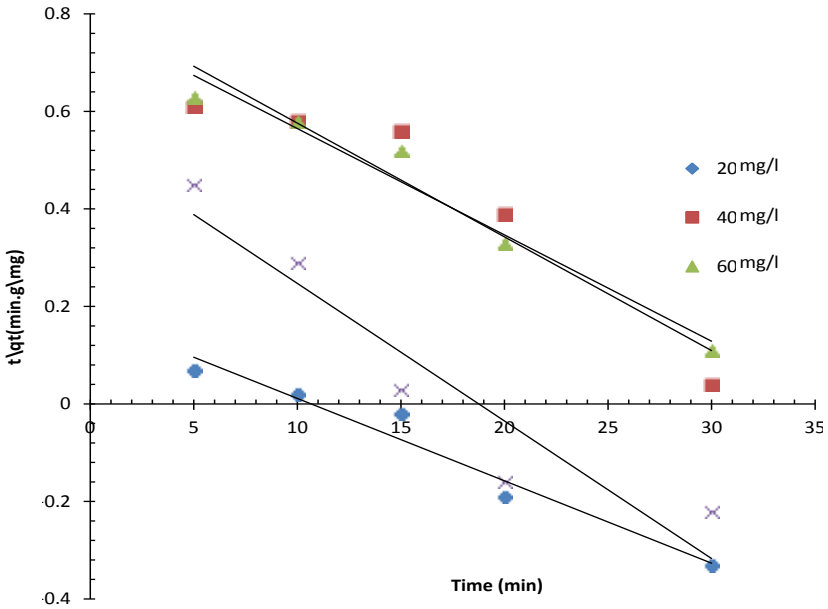

Figure 5. Pseudo-first order plot for methylene blue biosorption by *S. birrea* for different initial concentrations.

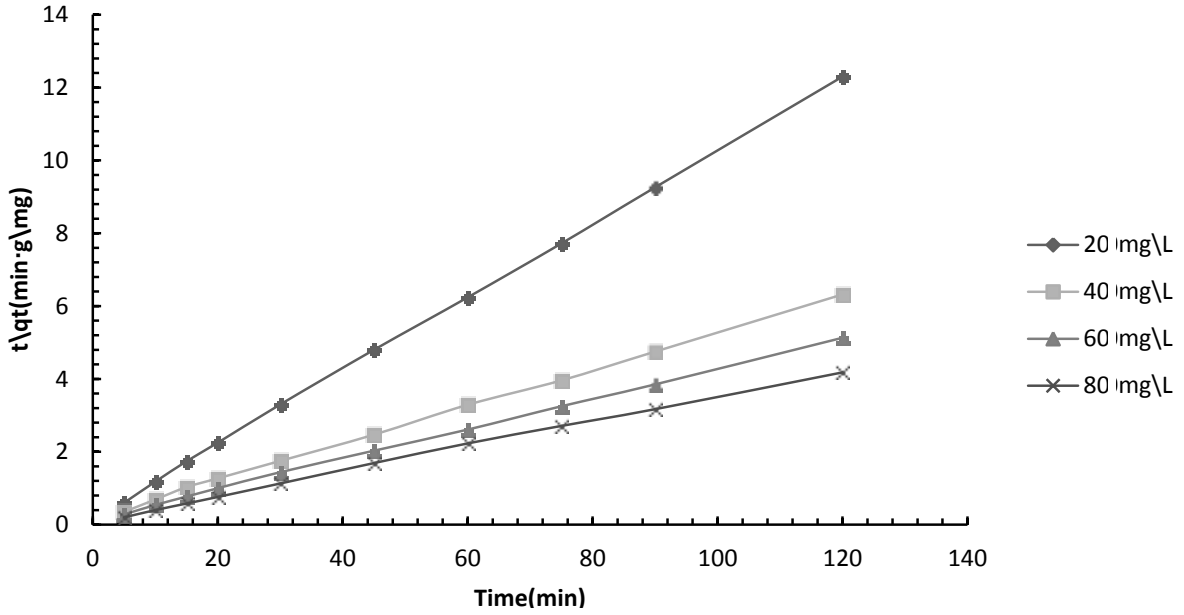

Figure 6. Pseudo-second order plot for methylene blue biosorption by *S. birrea* for different initial concentrations.

Table 3. Pseudo-first and pseudo-second order parameters for MB biosorption by *Sclerocarya birrea* fruit seed shell powder at different initial concentration.

Initial concentration	Pseudo-first order			Pseudo-second order		
($mg \cdot L^{-1}$)	k_1	q_e ($mg \cdot g^{-1}$)	R^2	k_2	q_e ($mg \cdot g^{-1}$)	R^2
20	0.0169	9.547	0.9580	0.11861	9.900	0.9998
40	0.0233	18.247	0.8933	0.1971	19.646	0.9993
60	0.0218	22.148	0.9622	0.1435	24.096	0.9995
80	0.0282	25.562	0.8897	0.0527	27.933	0.9998

Conclusion

The study demonstrated that *S. birrea* was a potentially effective biosorbent for the removal of methylene blue from aqueous solutions. A maximum adsorption capacity of 27.690 mg g^{-1} was achieved at an optimum pH 8. Given the abundant availability of the *S. birrea* trees in Southern Africa, the seed fruit shell can be used as a source of low cost biosorbent.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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African Journal of Biotechnology

Full Length Research Paper

The effects of plant growth regulators on *in vitro* gynogenic embryo formation in onion (*Allium cepa* L.)

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Different combinations of plant growth regulators were tried on *in vitro* gynogenesis by flower bud and ovary culture for two open pollinated Turkish onion cultivars, "Bayram 1" and "Yakut" and a synthetic population of "OH-1", which serves as a responsive control for extraction of gynogenic haploids of onion for two years (2011 and 2012). In 2011, modified Dunstan and Short (BDS) medium supplemented with 1 and 2 mg L^{-1} of 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BAP) and their combinations were used. Taking into account the first year results, combinations of 2 mg L^{-1} 2,4-D and 1 and 2 mg L^{-1} of BAP were used in second year experiment. Auxin (2,4-D) and cytokinin (BAP) combined together induced callus development in two onion cultivars (Bayram 1 and Yakut), but haploid plants were obtained only from "OH-1" with 2 mg L^{-1} of 2,4-D and 1 mg L^{-1} of BAP combination. Results showed that genotype is more important factor for inducing *in vitro* gynogenesis than plant growth regulators.

Key words: *Allium cepa* L., gynogenesis, haploidy, onion.

INTRODUCTION

In vitro techniques are important in obtaining haploid plants and decreasing the duration of onion breeding studies. There are different methods for inducing *in vitro* haploid production in onions, but only gynogenesis has been reported to be successful in *Allium* (Muren, 1989; Campion and Alloni, 1990; Keller, 1990a, b; Mukhambetzhanov, 1997; Javornık et al., 1998; Bohanec and Jakse, 1999; Hassandokht et al., 2000; Jakse et al., 2002; Szulc et al., 2002; Alan et al., 2003, 2004; Musial et al., 2005; Geoffriau et al., 2006; Sulistyaningsih et al., 2006; Forodi et al., 2009). In recent studies, ovary or flower bud culture was effective both for embryo yield and less labor for inducing gynogenesis in onions (Fayos et al., 2015). Although, gynogenesis induced haploidy, low success rate of variety, mother plant genotype and growing conditions, *in vitro* production media and supplementation plant growth regulators are the main effective factors (Anandhan et al., 2014; Mishra and Goswami, 2014). The availability of laboratory protocols for onion double haploid production represents a unique opportunity to have completely homozygous and stable inbred lines. To include gynogenesis protocols to

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breeding programs, genotype and optimization of different labaratory conditions are important. Gynogenesis frequency of Turkish onion cultivars has not yet been evaluated. For this reason, the present study aims to obtain haploid plants via flower bud or ovary culture supplemented with plant growth regulators by combining auxin (2,4 D) and cytokinin (BAP) in two Turkish onion cultivars.

MATERIALS AND METHODS

Plant material

Two onion cultivars, one with brown shell color (Bayram 1) and the other, red shell color (Yakut) were used as genetic material in both experimental years. 'Onion haploid (OH) -1, a long-day synthetic population intended to serve as a responsive control for extraction of gynogenic haploids of onion (Havey and Bohanec, 2007), was used as control in the second experimental year. Seeds of OH-1 were kindly provided by Dr. Michael J. Havey (The University of Wisconsin- Madison, USA). Bulbs of three varieties were planted in Vegetable Research and Application Garden of Horticulture Department of Agriculture Faculty, University of Ankara in both experimental years. Drip irrigation system was used for the irrigation of plants. In order to encourage the development of mother plants, 350 to 400 g L^{-1} of fertilizer (Novachem 18-18-18+ME) was applied with drip irrigation.

In vitro culture

Dunstan and Short (BDS) medium was used for induction of gynogenic embryos (Keller, 1990a; Bohanec and Jakse, 1999; Puddephat et al., 1999; Alan et al., 2003, 2004; Musial et al., 2005; Cho et al., 2006; Fayos et al., 2015). BDS medium was supplemented with 1 to 2 mg L^{-1} of 2,4-dichlorophenoxyacetic acid (2,4-D) and 1- 2 mg L^{-1} of 6-benzylaminopurine (BAP) and both were used in the first experimental year (2011). 2 mg L^{-1} of 2,4-D and 1 mg L^{-1} and 2 mg L^{-1} of BAP were used in induction medium in the second year. All media were adjusted to pH 6.0 and were solidified with 7.2 g L^{-1} Phytagar (Bohanec and Jakse, 1999; George et al., 2008). To increase the efficiency of embryo yield *in vitro* gynogenesis, 5 and 10% of sucrose doses were used as carbohydrate source in induction medium in the first experimental year (Muren, 1989; Bohanec and Jakse, 1999; Judkevieiene et al., 2005; Musial et al., 2005). In the second year, according to the rate of callus development of explants 5% sucrose dose was preferred. All media were sterilized by autoclaving for 20 min at 121°C.

To determine the more effective method for gynogenesis induction, both flower bud culture and ovary culture were used for two onion cultivars cv. "Bayram 1" and cv. "Yakut" in the first experimental year. According to the results of the first year experiments, only flower bud culture was used in 2012. Whole umbels or individual flower buds were dipped in 70% ethanol for 3 min, sterilized in 15% clorox (0.9% sodium hypochlorite) + 0.1% Tween-20 for 30 min. They were stirred and rinsed three times with sterile double distilled water.

After cutting, stalks of flower buds and isolated ovaries were placed in Petri plates containing 20 mL of induction medium (25 explants/plate) using a laminar flow cabinet in sterile conditions. Petri plates were sealed with stretch film and cultured at 25°C under cool white fluorescent with 16:8 (LD) h photoperiod (Puddephat et al., 1999; Alan et al., 2003; Sulistyaningsih et al., 2006). Developing explants were transferred to B1 medium supplemented with 1 mg L^{-1} of NAA (naphthaleneacetic acid) and 2 mg L^{-1} of 2iP (ısopentyladenine) and 100 g L^{-1} sucrose for 6 to 7 weeks after initial culture on BDS medium (BDS/B1). Emerging plantlets or excised gynogenic plantlets were transferred to plates of elongation medium (EM). EM is a modified BDS medium containing half strength major and minor salts, without growth regulators and 30 g L^{-1} sucrose (Alan et al., 2003).

In vitro observations and ploidy analysis

After one week of planting, callus formation, shoot and root formation were determined in cultures. Ploidy levels of gynogenic plantlets were determined by flow cytometry analysis using material from the youngest leaves of *in vitro* plants (Arumuganathan and Earle, 1991; Puddephat et al., 1999; Alan et al., 2003).

Acclimatization of *in vitro* plants

In vitro plantlets with 3 to 4 leaves and growing in solid M4 medium were transplanted in 7.5 cm pots, covered with plastic bags for several days and transferred to a growth chamber set as described previously for 6 to 16 weeks. The bags were punctured at several points after 1 week to help plant acclimatization and were removed at the end of 2 weeks (Alan et al., 2003).

Statistical analysis

The following variables were calculated: Number of explants forming callus (number/petri), rate of callus formation (%), number of explants forming roots and shoots (number/petri), rate of forming roots and shoots (%). All experiments were established according tofactorial designs in order to reveal the relationship between "genotype × culture method" and "genotype × medium composition". Tukey's HSD (Honest Significant Difference) test was used to compare means of the different treatments. Analysis was made with JMP software package version 5.0.1.

RESULTS AND DISCUSSION

A total number of 22500 flower buds and 22500 ovaries from the two cultivars (Bayram 1 and Yakut) in 2011 and 20 250 flower buds from "Bayram 1" and "Yakut" and 1125 flower buds from "OH-1" in 2012 were cultured *in vitro*. Flowers opened and ovaries started swallowing after 4 to 6 days of culture. Flower buds and ovaries from onion cultivars developed callus on the flower base after 2 to 3 weeks.

The effect of culture method

First year experiment results showed that ovary culture was the least efficient and the most time-consuming procedure. The highest callus formation rate (36.28%) was obtained from flower bud culture in cv. Yakut (Table 1). So, both higher callus formation rate and less labor for inducing it only used flower bud culture method in the second experimental year.

Effect of media composition

The effects of interaction between "genotype" and "media

Table 1. The effects of culture method on callus formation, roots and shoots formation in two onion cultivars in the first experiment year.

Explant development	Cultivar			
	Bayram 1		Yakut	
	Flower bud culture	Ovary culture	Flower bud culture	Ovary culture
Number of explants forming callus	4.23d	5.75c	9.07a	6.72b
Rate of callus formation (%)	16.92	23.00	36.28	26.88
Number of explants forming roots and shoots	151.00	673.00	1553.00	1403.00
Rate of forming roots and shoots (%)	11.92	42.27	57.22	58.90

*Different letters denote significant differences between the cultivars (p≤0.05).

Table 2. The effects of media composition on callus formation, roots and shoots formation in two onion cultivars in the first experiment year.

Media composition	Cultivar			
	Bayram 1		Yakut	
	Number of explants forming callus	Rate of callus formation (%)	Number of explants forming callus	Rate of callus formation (%)
0 mg L^{-1} 2,4D + 0 mg L^{-1} BAP	3.20^{c}	12.80	3.64^{c}	14.56
1 mg L^{-1} 2,4D + 1 mg L^{-1} BAP	4.20^{c}	16.80	6.50^{b}	26.00
1 mg L^{-1} 2,4D + 2 mg L^{-1} BAP	6.30^{b}	25.20	10.67^{a}	42.68
2 mg L^{-1} 2,4D + 1 mg L^{-1} BAP	4.26^{c}	17.04	7.07^{b}	28.28
2 mg L^{-1} 2,4D + 2 mg L^{-1} BAP	7.02^{b}	28.08	11.58^{a}	46.32
Media composition	**Number of explants forming roots and shoots**	**Rate of forming roots and shoots (%)**	**Number of explants forming roots and shoots**	**Rate of forming roots and shoots (%)**
0 mg L^{-1} 2,4D + 0 mg L^{-1} BAP	42	11.02	191	37.23
1 mg L^{-1} 2,4D + 1 mg L^{-1} BAP	0	0.00	413	50.12
1 mg L^{-1} 2,4D + 2 mg L^{-1} l BAP	164	32.41	505	58.11
2 mg L^{-1} 2,4D + 1 mg L^{-1} BAP	311	43.56	939	66.55
2 mg L^{-1} 2,4D + 2 mg L^{-1} BAP	307	40.55	908	61.39

*Different letters denote significant differences between the cultivars (p≤0.05).

composition" on callus formation in the induction medium for flower buds and ovaries taken from "Bayram 1" and "Yakut" were statistically significant. Also, callus formation was observed in induction medium that have no auxin and cytokinin. Rate of callus formation increased with increasing doses of auxin and cytokinin (Table 2). The highest callus formation rate was obtained from medium supplemented with 2 mg L^{-1} of 2,4-D and 2 mg L^{-1} of BAP in "Yakut" (46.32%) and "Bayram 1" (28.08%). Increasing the doses of auxin and cytokinin also increased the root and shoot development of explants. Root and shoot formation rate ranged between 66.55 and 11.02%. The highest rate of root and shoot formation were obtained from medium supplemented with 2 mg L^{-1} of 2,4-D and mg L^{-1} of BAP in "Bayram 1" (43.56%) and "Yakut" (66.55%).

One plantlet developed from callus of "Bayram 1" and 114 plantlets of "Yakut" have continued their development in elongation medium (EM). 41 plants of developed plantlets have 3 to 4 leaves of "Yakut" were planted in pots to acclimatize to the outside conditions. Unfortunately no plants were alive (Figure 1).

According to the results of the first year experiment, 2 mg L^{-1} 2,4-D + 1 mg L^{-1} BAP and 2 mg L^{-1} 2,4-D + 2 mg L^{-1} BAP were used in induction medium in the second experimental year. The highest callus formation rate was obtained from BDS medium supplemented with 2 mg L^{-1} of 2,4-D and 2 mg L^{-1} of BAP in "Yakut" (50.84%). Also, the lowest callus formation rate was obtained from medium containing no auxin and cytokinin for all onion cultivars. The number of roots and shoots emerging from the callus was affected by auxin-cytokinin concentrations (Table 3). The highest root and shoot formation rate (75.23%) was obtained from medium supplemented with 2 mg L^{-1} of 2,4-D and 2 mg L^{-1} of BAP in "OH-1". Only 3 plantlets of "Yakut" continued their development in elongation medium but they could not live.

Gynogenic plantlets of OH-1 emerged directly from the ovules rupturing the ovary wall unlike other onion cultivars used in experiment. 16 gynogenic plantlets

Figure 1. a. Onion umbels at the time of harvest. **b,** Flower buds used in *in vitro* culture. **c,** Callus formation in flower bud culture. **d,** *İn vitro* plantlets continuing development in elongation medium. **e,** Plants in pots to acclimatize to the outside conditions.

obtained by flower bud culture were transferred to elongation medium. But, only 3 plantlets could be kept alive in this medium. Flow cytometry used to analyze the ploidy levels of healthy gynogenic plants have 3 to 4 leaves. One of the three plants analyzed was haploid; one was diploid and one plant was mixoploid (haploid and diploid cells) (Figure 2).

Different factors such as genetic factors, including cultivar, donor plant genotype and geographic origin are thought to be the most important for the success of gynogenesis induction (Campion et al., 1992; Bohanec and Jakse, 1999; Chen et al., 2011; Anandhan et al., 2014). Even if the studies about producing *in vitro* gynogenic haploid plant all conditions are fulfilled, there is no change of success because the species has no haploidy frequency. Therefore, rate of gynogenic haploid embryos, embryo quality and regeneration of plants from embryos obtained by gynogenesis varies according to species and varieties (Keller and Korzun, 1996; Michalik et al., 2000; Alan et al., 2004; Judkevieiene et al., 2005; Palmer and Keller, 2005; Reed, 2005; Cho et al., 2006; Kim et al., 2007; Chen et al., 2011; Murovec and Bohanec, 2012). Therefore, it is necessary to determine the gynogenesis frequency of the study's onion genotypes in future studies. The introduction genotypes identified as high gynogenic capacity in work conducted in other countries of varieties will be important to demonstrate the gynogenic potential of the study's genotypes. Haploid plants could not be obtained from Bayram 1 and Yakut onion cultivars in both experimental years in this study but, haploid plant from OH-1 obtained from Prof. Dr. Michael J. Havey (University of Wisconsin) was produced; it served as a responsive control for extraction of gynogenic haploids of onion (Havey and Bohanec, 2007). Due to the low number of plants and flower buds, 1125 flower buds of OH-1 were cultured in experiment.

Table 3. The effects of media composition on callus formation, roots and shoots formation in onion cultivars in the second experiment year by flower bud culture.

Cultivar	Media composition					
	0 mg L^{-1} 2,4-D +0 mg L^{-1} BAP		2mg L^{-1} 2,4-D +1 mg L^{-1} BAP		2 mg L^{-1} 2,4-D +2 mg L^{-1} BAP	
	Number of explants forming callus	Rate of callus formation (%)	Number of explants forming callus	Rate of callus formation (%)	Number of explants forming callus	Rate of callus formation (%)
Bayram 1	4.94^{ef}	19.76	10.94^{b}	43.76	11.32^{b}	45.28
Yakut	5.63^{de}	22.52	11.81^{b}	47.24	12.71^{a}	50.84
OH-1	3.92^{f}	15.68	6.76^{cd}	27.04	7.06^{c}	28.24
	Number of explants forming roots and shoots	Rate of forming roots and shoots (%)	Number of explants forming roots and shoots	Rate of forming roots and shoots (%)	Number of explants forming roots and shoots	Rate of forming roots and shoots (%)
Bayram 1	0	0.00	0	0.00	0	0.00
Yakut	0	0.00	54	3.40	33	1.93
OH-1	0	0.00	32	31.68	79	75.23

*Different letters denote significant differences between the cultivars ($p \leq 0.05$).

So only 1 haploid, 1 diploid and 1 mixoploid plant were obtained. Havey and Bohanec (2007) found an average rate of 12% of gynogenic capacity of OH-1 when the mother plant was grown in controlled conditions. Gynogenesis response of onion material depended on the culture media used. Also, media composition request to obtain gynogenic haploid plant in species and varieties of *Allium* may be different (Mukhambetzhanov, 1997; Murovec and Bohanec, 2012; Yarali and Yanmaz, 2013). Plant growth regulators added to the culture medium have also significant effect on gynogenesis (Mukhambetzhanov, 1997; Bohanec, 2009; Murovec and Bohanec, 2012). It is reported that different doses of 2,4-D as auxin and BAP as cytokinin are added in nutritional medium in studies of gynogenic haploid induction in onion (Campion et al., 1992; Geoffriau et al., 1997; Martinez et al., 1997; Alan et al., 2003, 2004; Bekheet, 2004; Musial et al., 2005; Reed, 2005; Sulistyaningsih et al., 2006; Bohanec, 2009; Forodi et al., 2009; Chen et al., 2011; Yarali and Yanmaz, 2013). Because of effective doses of auxin and cytokinins particularly affected by genotype, it cannot be given specific values for the concentration of auxin-cytokinin in medium (Keller and Korzun, 1996; Chen et al., 2011; Murovec and Bohanec, 2012; Yarali and Yanmaz, 2013). In this study, 1 and 2 mg L^{-1} of 2,4-D and BAP combinations had been tried in induction medium. Explant development had been very little or not in induction medium that have no auxin and cytokinin. Whereas it was found that the number of explants increased with increasing doses of auxin and cytokinin. Combinations of 2+1 mg L^{-1} of 2,4-D+BAP and 2+2 mg L^{-1} of 2,4-D+BAP were more effective than other doses on growing flower buds and ovaries in onion cultivars (“Bayram 1” and” Yakut”) in both experimental years. Similar results were obtained from OH-1 onion cultivar. Also gynogenic haploid plant was obtained from medium supplemented with 2 mg L^{-1} of 2,4-D and 1 mg L^{-1} of BAP. We could not obtain much number of haploid plants as in previous studies (Campion et al., 1992; Martinez et al., 1997; Michalik et al., 2003).

Conclusion

This study aimed to obtain haploid plants via gynogenesis with onion cultivars, particularly from Turkey. But the methods used in previous study that was successful were not in the results of our study. Even so, this method actively used in other country onion breeding work is required to be used in the breeding programs. For this reason, this study provides guidance for researchers that determine the capacity of genotype that serves as a responsive control for extraction of gynogenic haploids of onion cultivars.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

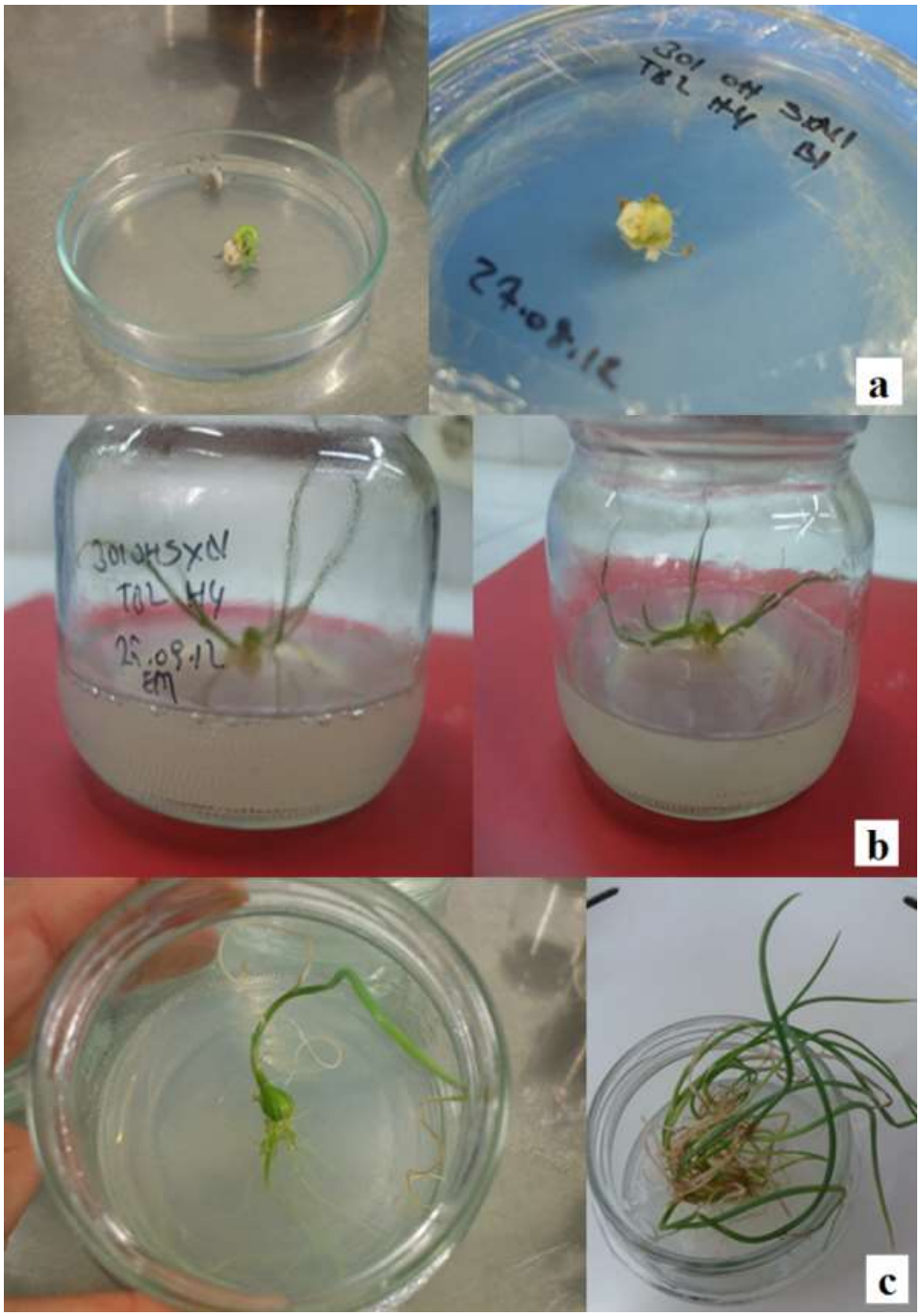

Figure 2. a, Emergence of a gynogenic plantlet from flower buds. **b,** *In vitro* plant in elongation medium (EM). **c,** Haploid plantlet (on left) and diploid onion plantlet (on right).

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Full Length Research Paper

Selection of loose-leaf lettuce breeding lines based on non-parametric indexes

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Non-parametric selection indexes (that is, require no estimation of parameters) can be used to help the selection process in the final stage of genetic enhancement. In this context, the objective of this study was to evaluate the efficiency of non-parametric selection indexes to choose promising lineages of loose-leaf lettuce, so that genetic gain of each lineage can be estimated and then registered in the Ministry of Agriculture. The indexes of Mulamba and Mock, Elston and Schwarzbach were used in the analysis. Ten genotypes of loose-leaf lettuce were evaluated, with eight of them being lineages (L_1, L_2, L_3, L_4, L_5, L_6, L_7, L_8) and two commercial cultivars (Vanda and Vera). The experiments occurred in six different locations of cultivation during the autumn and winter seasons of 2014. Experimental delineation was composed of random blocks, with four repetitions and the evaluated characteristics were: total production, commercial production, number of leaves, plant volume and stem length. The effects of genotype (G), location (L), and G x L interaction were significant for all characteristics. Indexes were correlated to classify the genotype. The Mulamba and Mock index stood out because it enabled good direct gains for the evaluated characteristics and because of its easy construction. Therefore, this index is recommended for selection of loose-leaf lettuce genotypes in the stage of cultivar recommendation in different locations. As the best lineages of loose-leaf lettuce were L_2, L_3, L_7 and L_8, these lineages have been considered promising and are recommended for registration.

Key words: Agronomic performance, *Lactuca sativa,* nonlinear indexes, spearman correlation.

INTRODUCTION

Lettuce is one of the most consumed vegetables in the world and its consumption has grown yearly. This is mainly due to new dietary habits that the population has adopted, which includes lettuce as an indispensable ingredient of its meals. In this context, market demand for high quality lettuce is already a reality, since consumers

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have become more selective and critical when choosing their foods. The Lettuce Breeding Program of the University of the State of São Paulo (UNESP-FCAV) has been active since 2003 and has worked on two different matters. The first regards *Bremia lactucae* L. breed identification in lettuce production regions of the State of São Paulo involves annual monitoring (Braz et al., 2007; Castoldi et al., 2012; Galatti et al., 2012; Nunes et al., 2016; Souza et al., 2011). The second is related to crossing of loose-leaf lettuce genitors and differentiating lettuces of *Dm* genes resistant to *B. lactucae*, with the intention of obtaining new lineages with positive agronomic and *B. lactucae* resistant traits (Castoldi et al., 2014). Currently, the program works on promising lineages that can be evaluated in different cultivation locations.

Agronomic yield assays performed in different locations which use different cultivation systems is an important stage of genetic enhancement, because necessary agronomic and productive characteristics are tested so that new lineages can be recommended in the future. The use of non-parametric selection indexes (indexes that do not require parameter estimations) can help in the lineage selection process when getting to the final stage of genetic enhancement. Non-parametric indexes have the intention of classifying genotypes in a simple way (Garcia and Souza Junior, 1999).

A few studies have reported the efficiency of non-parametric selection indexes in the beginning of progenies enhancement programs (Neves et al., 2011; Oliveira et al., 2008), as well as in advanced lineage selection stages (Marinho et al., 2014; Vittorazzi et al., 2013). However, studies using selection indexes on lettuce are still scarce. In this context, the objective of this study was to verify the efficiency of non-parametric selection indexes in choosing promising lineages of loose-leaf lettuce in order to register them in the Brazilian Ministry of Agriculture and to estimate genetic gains based on the same indexes.

MATERIALS AND METHODS

The experiments were installed during autumn and winter of 2014, in the cities of Monte Alto, São Simão, Aramina, Mogi das Cruzes, Biritiba Mirim, and Salesópolis - São Paulo State (Brazil). Experimental design was based on random blocks with six experimental groups and four repetitions. Treatments composed of 10 genotypes (8 lineages and the 2 commercial cultivars Vanda and Vera) and six (6) locations (Monte Alto, São Simão, Aramina, Mogi-das-Cruzes, Biritiba-Mirim, and Salesópolis). Each plot composed of 28 plants distributed in 4 rows with each row 1.75 m long and plants were spaced at 0.25 m intervals, but only the 6 central plants of each group were evaluated. The evaluated lineages came from the initial crossings of JAB 4-13-7 (male genitor, possessor of the *DM*18 gene of *B lactucae* resistance) with commercial cultivars: Argelis (A) – possessor of the *B. lactucae* resistance factor R-38, Vanda (V), Venerada (Vn) and Solaris (S) (all female genitors). This lineage resisted the *B. lactucae* breeds that occurred in the State of São Paulo (Castoldi et al., 2014).

The agronomic evaluated characteristics were: total production in g plant $^{-1}$ (TP), commercial production in g plant $^{-1}$ (CP), number of leaves per plant (NL), volume of the plants (cm^3 planta^{-1}) (V) and stem length in cm (SL). These measurements were done at harvest point (45 days after transplantation). Total production (TP) was obtained by calculating the average of the fresh mass in 6 plants (without extracting the old leaves). Commercial production (CP) was obtained by measuring the average weight of the fresh mass in 6 plants after removal of old leaves and of spare stem. NL represents the total number of leaves that reached the length of at least 3 cm in each plant. Volume (VOL) was calculated by multiplying the diameters of extremities, D_1 and D_2, and the height (h) of 6 plants from each of the portions (groups). In order to calculate V, the volume formula of 2 diameter used ellipsoid was: $V = 4/3\ \pi\ (D_1/2)\ (D_2/2)\ h$. Stem length (SL) was measured using a graduated ruler. The obtained data were initially submitted to the normality test and homogeneity of residual variances, and normality of the data was detected. Subsequently, data were submitted to statistical analysis of variance by location (environment), in which the variation sources were considered random. To do so, the statistical genes computer program (Cruz, 2013) was used. The formula adopted for joint analysis was:

$$Y_{ijk} = m + g_i + a_j + b/a_{jk} + ga_{ij} + e_{ijk}$$

Where, Y_{ijk} is the mean phenotypic value of the group; m is the average; g_i is the fixed effect of the i^{-th} genotype; b/a_{jk} is the effect of k repetition in location j; ga_{ij} is the interaction effect of genotype i in location j and e_{ijk} is the experimental error. The selection indexes were constructed following Mulamba and Mock (1978), Elston (1963) and Schwarzbach (1972) and Wricke and Weber (1986). The expected gain by direct selection in the i^{-th} character was estimated based on selection indexes studied through the following expression:

$$GS_i = (X_{si} - X_{oi})h_i^2 = DS_i h_i^2 \text{ and } GS_i(\%) = \frac{GS_i}{X_{oi}}\ x\ 100$$

Where, X_{si} is the average of selected samples for characteristic i; X_{oi} is the original average of the population; DS_i is the selection differential carried out in the population; and h_i^2 is the heritability of characteristic i. In order to compare lineage classification using the indexes of Mulamba and Mock (1978), Elston (1963) and Schwarzbach (1972), the Spearman correlation between them was obtained.

RESULTS AND DISCUSSION

Significant effects were verified for genotypes, locations and genotype x location (GXA) for all evaluated characteristics (Table 1). For the Monte Alto location (Table 2), the Mulamba and Mock (1978) indexes selected lineages L_2, L_3 and L_7, whereas the Elston index (1963) indicated lineages L_2, L_3 and L_4 and the Schwarzbach index (1972) chose lineages L_4, L_7 and L_8. Lineages L_2 and L_3 were indicated by both the I_{MM} and I_E indexes. Index I_{MM} presented the lowest rank; D_i represented shortest distance from the genotype to the ideotype and I_E the highest value. Lineage L_4 indicated by indexes I_E and D_i, also presented higher results for TP, CP and V. These characteristics are relevant for lettuce cultures since the consumer market demands bulky plants and good-looking leaves. Thus, lineages L_2, L_3 and

Table 1. Variation analysis chart of 6 random block experiments with ten loose-leaf lettuce genotypes, conducted in 6 different locations in autumn-winter, 2014.

FV	GL	Average square				
		TP	CP	NL	V	SL
Block/location	18	8102.57	5994.67	5.50	79301734.96	0.75
Genotype (G)	9	25850.03**	2009.27**	114.21**	341670068.37**	11.74**
Location (L)	5	486894.78**	423561.97**	360.46**	1778274902.9**	26.73**
G X L	45	4605.83**	3705.96**	6.17*	29966030.15**	0.89**
Error	162	2589.83	2055.57	4.10	14569571.94	0.28
Average		381.55	347.52	25.98	25546.68	4.53
CV		13.34	13.05	7.79	14.94	11.76

FV, Sources of variation; GL, degrees of freedom; TP, total production (g/plant); CP, commercial production (g/plant); NL, number of leaves; V, plant volume (cm³/plant); SL, stem length (cm). **, * relative to 1 and 5% probability, respectively, by the F test. CV = coefficient of variation.

Table 2. Indexes: Mulamba and Mock (I_{MM}), Elston (I_E) and Schwarzback (D_i), applied to the characteristics total production (g/plant); commercial production (g/plant); number of leaves; plant volume (cm³/plant) and stem length (cm) for 10 genotypes of loose-leaf lettuce in 6 different locations in the autumn and winter seasons of 2014.

Genotypes	Monte Alto-SP			São Simão-SP		
	I_{MM}	I_E	D_i	I_{MM}	I_E	D_i
L_1	36	7.95	5.00	32	8.09	4.19
L_2	**19**	**9.90**	3.55	**19**	**9.20**	4.22
L_3	**20**	**9.83**	3.50	**21**	8.87	3.79
L_4	21	**9.48**	**3.39**	**21**	8.53	**3.27**
L_5	39	0.00	5.96	32	0.00	4.43
L_6	24	9.22	3.64	27	8.78	3.45
L_7	**20**	9,26	**3.29**	**18**	**9.07**	**2.53**
L_8	31	9.04	**3.36**	**21**	**8.95**	**3.14**
‘Vanda’	31	9.35	4.32	45	0.00	6.32
‘Vera’	39	0.00	5.85	39	0.00	5.57

Genotypes	Salesópolis-SP			Biritiba Mirim-SP		
	I_{MM}	I_E	D_i	I_{MM}	I_E	D_i
L_1	30	5.67	4.09	27	**8.96**	3.65
L_2	39	4.82	4.44	35	8.69	4.40
L_3	24	7.25	2.93	35	8.54	4.15
L_4	32	6.50	3.82	34	8.46	3.82
L_5	26	0.00	5.71	23	0.00	3.20
L_6	**12**	**7.94**	**1.63**	**12**	**9.46**	**2.54**
L_7	**14**	**7.50**	**2.50**	**11**	**9.09**	**1.85**
L_8	**16**	**7.67**	**2.06**	**17**	8.79	**2.08**
‘Vanda’	41	0.00	4.60	39	7.34	5.26
‘Vera’	41	0.00	5.87	42	0.00	6.85

Genotypes	Mogi das Cruzes-SP			Aramina-SP		
	I_{MM}	I_E	D_i	I_{MM}	I_E	D_i
L_1	24	**8.78**	4.39	23	**9.09**	3.18
L_2	37	7.78	4.80	**21**	**9.13**	**2.86**
L_3	27	8.42	4.18	**21**	**9.35**	3.84
L_4	**23**	8.52	**3.46**	29	7.70	3.65
L_5	34	0.00	5.06	37	0.00	4.68

Table 2. Contd.

L_6	27	8.18	3.56	36	5.38	4.50
L_7	**9**	**9.22**	**2.10**	**21**	8.08	**2.48**
L_8	**13**	**8.80**	**1.86**	**12**	8.89	**1.76**
'Vanda'	37	8.17	4.65	32	7.43	4.25
'Vera'	44	0.00	6.69	43	0.00	5.69

Results in bold indicate the selected genotypes using the index in the location.

Table 3. Estimates of genetic gains in percentage, by the indexes of selection of Mulamba and Mock (IMM), Elston (IE) and Schwarzback (Di) applied to the characters total production (TP) (g / plant); commercial production (CP) (g / plant); number of leaves (NL); plant volume (V) (cm³ / plant); stem length (SL) (cm) for ten genotypes of crisphead lettuce, in six environments, in the fall-winter of 2014, based on the selection at each site..

Indexes	Monte Alto-SP						São Simão-SP					
	TP	CP	NL	V	SL	Total	PT	PC	NF	VOL	CC	Total
I_{MM}	10.13	9.09	3.16	14.98	9.49	46.85	5.28	4.88	4.23	7.75	3.16	25.30
I_E	8.34	6.85	-0.61	16.17	11.71	42.46	5.93	5.17	8.84	4.52	3.97	28.43
Di	2.98	3.32	3.95	-1.03	-1.82	7.40	3.78	3.05	9.17	0.58	-1.29	15.29

Indexes	Salesópolis-SP						Biritiba Mirim-SP					
	TP	CP	NL	V	SL	Total	TP	CP	NL	V	SL	Total
I_{MM}	9.07	10.96	7.33	1.66	-4.46	24.56	12.93	13.51	7.75	13.12	-2.13	45.18
I_E	9.07	10.96	7.33	1.66	-4.46	24.56	11.14	11.34	6.48	15.96	3.74	48.66
Di	9.07	10.96	7.33	1.66	-4.46	24.56	12.93	13.51	7.75	13.12	-2.13	45.18

Indexes	Mogi das Cruzes-SP						Aramina-SP					
	TP	CP	NL	V	SL	Total	TP	CP	NL	V	SL	Total
I_{MM}	6.05	6.06	10.50	6.51	-3.42	25.70	2.34	2.55	5.53	11.41	7.18	29.01
I_E	6.77	6.31	9.29	8.66	2.40	33.43	2.66	2.58	1.46	24.08	25.00	54.32
Di	6.05	6.06	10.50	6.51	-3.42	25.70	2.13	2.38	7.82	2.41	-1.60	13.14

L_4 are the most indicated for cultivation in Monte Alto, taking into consideration the group of characters evaluated.

The other lineages and commercial cultivars Vanda and Vera did not present satisfactory agronomic development. Their selection index results were low when compared to the lineages selected, which does not justify their cultivation in the Monte Alto region. Direct gains in Monte Alto, based on the adopted 30% selection intensity, were higher for V, with a 14.98 to 16.17% increase by the I_{MM} and I_E indexes. Additionally, higher total gains were obtained in all characteristics by the same indexes. The D_i index showed no satisfactory gains when compared to indexes I_{MM} and I_E, especially for TP and V (Table 3).

Rosado et al. (2012) found different results than the ones presented in the current study where they worked with simultaneous selection using selection indexes in progenies of sour passion fruit trees and verified the use of the Elston index which was not capable of distributing gains fitting for the purposes of that research. Thus, based on the results, it is possible to deduce that gains from index are subject to variation according to the greatness of the evaluated measurements. However, the same authors found that the index based on the additions of the Mulamba and Mock rankings was the most adequate, because it promoted balanced gain distribution. This result did support the results obtained in the current study.

In the region of São Simão (Table 2), indexes did not indicate the same lineages as the ones selected in Monte Alto. The Mulamba and Mock (I_{MM}) index identified 5 lineages: L_2, L_3, L_4, L_7 and L_8. On the other hand, the Elston index (I_E) indicated lineages L_4, L_7 and L_8, which was in agreement with the other 2 indexes that selected lineages L_2, L_7 and L_8. The Schwarz back index (D_i) also indicated lineages L_4, L_7 and L_8 for plantation in São Simão, which is also in agreement with the I_{MM} index.

The highest direct gain was in NL (9.17%), measured by D_i, however the highest gain values were for the I_E

(28.43%) and I_{MM} (25.30%) indexes. The I_E index showed the highest gain percentage for NL (8.84%) and the I_{MM} index presented the highest gain for V (7.75%) (Table 3). Terres et al. (2015), working to estimate genetic gains using different indexes in hybrid potato populations, verified that the use of indexes resulted in better selection gain estimations. One of the best indexes to use for genetic potato enhancement was the Mulamba and Mock (1978) index, which is in agreement with the obtained results. Similarly, Teixeira et al. (2012), using selection indexes in simultaneous component enhancement in the production of açaí fruits, also confirmed that the Mulamba and Mock (1978) index was the most efficient to estimate gains in production components.

In Salesópolis (Table 2), all indexes selected lineages L_6, L_7 and L_8 as the best genotypes. The selected lineages had the superior results for the set of evaluated characteristics, but especially stood out in TP, CP and NL when compared with the commercial tested cultivars. This revealed that the selected lineages had good aptitude in agronomic performance assays. Selection gains were highest for CP (10.96%) and SL reduction was -4.46%, which indicates that planting conditions in this region tend to stand out due to its commercial production. Total gains for all 3 indexes were lower than those obtained in other locations, such as in Biritiba Mirim (Table 3). In Biritiba Mirim (Table 2), indexes I_{MM} and D_i recommended the same lineages: L_6, L_7 and L_8. The I_E index indicated lineages L_6 and L_7 as well, differing therefore from the I_{MM} and D_i indices in the recommendation of L_1 lineage only instead of L_8. The highest obtained gains were for CP (13.51%) and V (13.12%), using the I_{MM} and D_i indexes. The highest total direct gains were observed by the I_E index (48.66%), which showed 15.96% of gain in V; its highest gain. However, using I_{MM} and D_i indexes, superior gains were obtained for TP, CP and NL and -2.13% reductions for SL (Table 3).

In Mogi das Cruzes (Table 2), both I_{MM} and D_i indexes indicated that lineages L_4, L_7 and L_8 were the best choices. I_E indicated lineages L_1, L_7 and L_8. Total direct gains for all characteristics were higher in the I_E index (33.43%) and NL and V especially stood out. However, the characteristic that had the highest gain was NL (10.5%), shown by I_{MM} and D_i indexes (Table 3).

Cities located in the “green belt” (Cinturão Verde) of São Paulo State (Salesópolis, Biritiba Mirim and Mogi das Cruzes) obtained similar results for the best lineages to plant. L_7 and L_8 were indicated as the best lineages to plant in the winter (Table 2). This relationship is due to similar climate and soil conditions found in this region of the State, which stimulate lineages to behave similarly. For cultivation conditions of Aramina (Table 2), the I_{MM} index indicated L_2, L_3, L_7 and L_8 as the best lineage genotypes to plant. On the other hand, I_E recommended lineages L_1, L_2 and L_3 and D_i selected L_2, L_7 and L_8. Total direct gains were highest when using the I_E index (54.32%), while the I_{MM} index enabled total gain of 29.01% and D_i had the worst total gains (Table 3).

In order to verify the relationship among the used indexes in the analysis of each location, Spearman correlation estimation was done, and significant correlations were observed. In Monte Alto, the observed correlations were: I_{MM} and I_E (0.902**), I_{MM} and D_i (0.734*) and between I_E and D_i ($0.549^{n.s}$); in São Simão, they were I_{MM} and I_E (0.898**), I_{MM} and D_i (0.802**) and between I_E and D_i ($0.631^{n.s}$); in Salesópolis, they were I_{MM} and I_E (0.874**), I_{MM} and D_i (0.842**) and between I_E and D_i (0.982**); in Biritiba Mirim, they were I_{MM} and I_E (0.730*), I_{MM} and D_i (0.980**) and between I_E and D_i (0.676*); in Mogi das Cruzes, they were I_{MM} and I_E (0.938**), I_{MM} and D_i (0.910**) and between I_E and D_i (0.893**); and in Aramina, they were I_{MM} and I_E (0.884**), I_{MM} and D_i (0.931**) and between I_E and D_i (0.702*). Significant correlations between I_{MM} and I_E and between I_{MM} and D_i occurred, while correlations between I_E and D_i for Monte Alto and São Simão were the lowest within all results. Therefore, one can deduce that the I_{MM} selection index may be best to use in advanced selections of lettuce lineages, due to its correlations with the other indexes and because of its easy application. Lessa et al. (2010), working with diploid hybrid selection of banana trees, based on non-parametric indexes, also verified strong correlations between Elston and Mulamba and Mock indexes, and between Mulamba and Mock and Schwarz back indexes, which were 0.76 and 0.63, respectively. Their results are in agreement with the results obtained in the research described here.

Conclusion

The Mulamba and Mock index enabled positive gains for the evaluated characteristics due to its strong correlations with the other studied indexes and because it can be easily obtained. Its use is recommended for loose-leaf lettuce genotype selection in the cultivar recommendation phase. Lineages L_2, L_3, L_7 and L_8 presented good productive potential, which improves the possibility of introducing new cultivars of loose-leaf lettuce, which positively respond to the climatic conditions of the autumn and winter seasons, in São Paulo State.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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