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Research Article

Heterosis in bread dough fermentation using chimeric genomes of *Saccharomyces cerevisiae*

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Abstract

Hybridization is the crossing between two divergent lineage genomes that give offspring harboring an admixture of both parental strains. This study aimed to generate chimeric genomes of yeast hybrids to enable them to survive under sucrose stress and play a prominent role in the bread making industry. In this investigation, three parental strains and two progenitor hybrids were evaluated for the leavening ability of bread dough under four concentrations of sucrose. For optimal fermentation rates, the use of appropriate yeast genotypes is required. Therefore, this study focused on the positive correlation detected between the weight of fermented bread dough and sugar concentrations as obtained by P_1, H_2, and H_1 genotypes, when the fermentation medium is warm water. Positive correlation was also obtained by P_2, and P_3 genotypes in the relationship between the increase in fermented bread dough weight and sucrose concentrations. In this association between sucrose concentrations and the increase in fermented bread dough weight, the P_2 and H_2 genotypes appeared a strong correlation between both variables. Meanwhile, hybrid genotype H_2 exhibited positive heterosis in the increase of bread dough weight at all sucrose concentrations ranging between 46.81 to 145.45%. The results indicated that P_2 and hybrid H_2 genotypes were tolerant to sucrose concentrations. Sucrose tolerant genotypes provide a useful genetic background for further application concerning the functionality of yeast as a leavening agent in the fermentation of bread dough.

1. Introduction

Baker's yeast or *Saccharomyces cerevisiae* is a species of yeast that has two mating types a and α which are primitive aspects of sex differentiation that lead to genetic recombination to produce novel combinations of chromosomes. Two haploid cells from the opposite mating types can mate to develop a diploid state, which can either sporulate to produce another generation of haploid cells or continue to exist in diploid cells. Mating has been demonstrated by geneticists as a tool to combine genes or proteins at will [1]. If there were abundant availability of nutrients, *Saccharomyces cerevisiae* reproduces via mitosis as diploid cells. However, as nutrients become scarce, the cells undergo meiosis to form haploid spores [2]. The *Saccharomyces cerevisiae* genome consists of about 12.156.677 base pairs distributed on about 6275 genes [3]. These genes are compactly organized on 16 chromosomes. About 5800 of these genes are believed to be functional [4]. The ability of cells to respond to environmental conditions was essential [5]. Sporulation and meiosis in diploid *Saccharomyces cerevisiae* were induced by starvation to nitrogen and fermentable carbon sources. Meiosis in the budding yeast, *Saccharomyces cerevisiae* is well-

generated asci containing four spores, each of which harbors a haploid genotype [6]. Spore generation starts at the beginning of meiosis II. This process is equivalent to the formation of buds in mitosis. Each spore grows around a nuclear lobe at which a haploid genotype becomes segregated. The cells respond to the limitation of carbon sources by generating an increased number of asci containing four haploid spores [7]. The external stress faced by yeast cells is growth inhibitory agents, starvation to one or more nutrients. These may drive yeast cells to leave the mitotic cycle to enter one of these specific states as sporulation, switching to pseudo hyphal growth generating elongated chains of cells or entering the stationary phase, where the aged cells undergo programmed cell death [8]. During starvation, activation of a common set of stress response genes was achieved regardless of when the substrates were limited [9]. The cells entered the stationary phase cell cycle (GO) as a consequence of nitrogen limitation, the supplement of glucose does not stimulate the renewal of growth [10]. The genome analysis of many wild-type strains of yeasts isolated from natural environments showed that they are diploid strains rather than haploid or polyploid [11]. Yeast cells have a heterozygous locus for the mating type which can respond to the changes in nutrient states in the environment in a variety of ways [12]. Starvation to a nitrogen source combined with the absence of a fermentable carbon source drives cells to enter meiosis and sporulation [13].

The loaf size of yeast-fermented products appears if the dough gives the yeast genotype a favorable environment for growth and gas release [14]. Genes fermenting activity is defined by the quantity of gas generated in the dough. The fermenting power depends on the α and β-amylase genes transforming starch into maltose in addition to the quality of the yeast genotype [15]. Therefore, approximately 95% of fermenting sugars are transformed into ethyl alcohol and CO_2, meanwhile, the residual 5% are transformed into superior alcohols, and organic acids, in addition to volatile compounds [16]. Usually, the word flour refers to wheat flour. If wheat flour was mixed with water, the gluten complex protein was formed. The development of gluten protein supports wheat dough with an elastic structure that facilitates the dough to be used in a variety of food types. This allows for generating gas bubbles in the dough structure, leading to the

development of a sponge-like texture in the end product [17]. Most types of bread are leavened with the yeast *Saccharomyces cerevisiae*, which is mixed with flour, salt, and warm water or milk [17].

A hybridization study based on mating between different genotypes was successful in the isolation of yeast hybrids [18]. From the study of [18], some recombinants that resulted from hybridization between *Saccharomyces cerevisiae* and *Saccharomyces rouxii* induced a higher dough-raising capability in the stimulation of bread dough fermentation than the original strains. Hybrids may exhibit unique phenotypic characteristics that are not necessarily intermediate between both parents in the progenitors. Hybridization of *Saccharomyces cerevisiae* with cryo-resistant mater strain produced improved offspring with leavening ability at low and high-sugar bread dough in addition to freeze-tolerant ability [19]. The *Saccharomyces cerevisiae* referred to as baker's yeast is a main leavening agent in the production of bread in its different forms [20]. Yeast cells use fermentable sugars containing dough to generate carbon dioxide and ethanol which are responsible for the leavening of dough at the fermentation phase [21]. The fermentation ability depends on the form of yeast cells, as well as the availability of fermentable sugars containing flour, as maltose results from starch hydrolysis [22]. The total value of mono and disaccharides containing flour differed with the mean of 4 mg per gram of flour. Sucrose is the main component of flour accounting for more than 50% of total soluble sugars [23]. The final gas volume of bread reached over 70% of the loaf volume [24]. The new bubbles are formed in dough during the agitation of dough ingredients. No new bubbles are formed during fermentation, but the volume of bubbles in dough can be increased via CO_2 production, which leads to increasing the volume of dough. Thus, the number of pores created at this stage. If the fermentation stage is delayed, the acid produced by alcohol oxidation leads to the production of sour-tasting products [25]. The amounts of free saccharides naturally contained in wheat flour range from 0.05% (glucose, fructose, and maltose) to 0.3% (sucrose and raffinose) [26]. Therefore, the main components

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Table 1. Sources and codes of *Saccharomyces cerevisiae* used in this study samples.

Sample code	Sample	Source or reference
P_1	Pakmaya	Pak Gida Uretim Ve Pazarlama A. S., Made in Turkey
P_2	Holw El-Sham	Holw El-Sham Company for Food Industries and Agriculture investment (S. A. E), 6 October City, Egypt
P_3	Dream	Dreem Mashreq Foods (S. A. E) New Borg El-Arab City, Alexandria, Egypt.

Table 2. Antifungal agents and their concentrations used for marking yeast strains.

Antifungal agents	Concentration	Abbreviation
Trefflucan (Flucanazole)	150 mg/ 10 mL distilled water	*Trf*
Flucoral	150 mg/ 10 mL distilled water	*Flu*
Gyano-Daktarin	20 mg/10 mL distilled water	*Gyn*
Benefits	250 mg/ 10 mL distilled water	*Ben*
Fungi can	150 mg/ 10 mL distilled water	*Fun*
Itracon (Itraconazole)	100 mg/ 10 mL distilled water	*Itr*

of fermentable sugars containing dough are generated from starch degradation by amylases [27]. The amount of damaged starch ranged from 5 to 8% in wheat flour [25]. Fructans and sucrose are hydrolyzed into fructose and glucose by invertase secreted by *S. cerevisiae* [28]. The purpose of this study is to investigate how different recombinants of baker's yeast hybrids can affect the leavening ability of bread dough under different sucrose concentrations.

2. Materials and methods

2.1. Yeast strains

Three commercial strains of Baker's yeast, *Saccharomyces cerevisiae*, were used in this study. These strains were derived from instant dry yeast purchased from the local market in Egypt, as shown in Table 1.

2.2. Culture media

Yeast extract peptone glucose (YEPG) medium contained yeast extract 1%, bactopeptone 2% and glucose 2%, solidified with 2% agar if needed, was used for growth and maintenance medium [29]. Cultivation of yeast cells was carried out in an aerobic condition using a 500 mL flask containing 250 mL of YEPG medium at 30°C. Yeast cells were collected after three days by centrifugation and then washed by twice by tap water to prepare 200 mL yeast cell suspension by water. This suspension contained 4-5% dry matter (W/V). The suspension was used to inoculate dough ingredients to determine leavening ability in terms of CO_2 production. Pre-sporulation medium was used in this study according to [30]. In addition, sporulation medium was used according to [31].

2.3. Isolation and purification of yeast strains

Saccharomyces cerevisiae strains were actually isolated from reactivated dried yeast cells from three resources of instant dry yeast using serial dilution technique to isolate single colonies which were picked up and purified, as well as microscopically examined according to [32].

2.4 Genetic marking

Yeast strains were subjected to antifungal drugs for genetic marking using a disk diffusion technique according to [33].

2.5. Antifungal agents

Antifungal agents are important tools in selecting the generating yeast hybrids. They allow efficient selection of *Saccharomyces cerevisiae* hybrids. They are alternatives to auxotrophic mutants used for selectable genetic marking. The yeast strains were genetically marked with six antifungal agents, as seen in Table 2. The genotyping of yeast strains used in this study after genetically marking with antifungal agents is shown in Table 3.

Table 3. Genetic markers in yeast strains used in this study.

Strain code	Genotype
P_1	Trf^- Flu^- Gyn^+ Ben^+ Fun^- Itr^-
P_2	Trf^- Flu^- Gyn^- Ben^+ Fun^- Itr^+
P_3	Trf^- Flu^- Gyn^- Ben^- Fun^- Itr^+

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2.6. Hybridization technique

One mL of yeast cell suspension grown for 24 hours at 30° was used for mating. The mating cells that carried the opposite genetic markers were mixed in the pre-sporulation medium for 24 hours at 30 ^{0}C and then transferred to the sporulation medium. The mated cells were incubated at 30 ^{0}C for 30 days on a sporulation medium. Single colonies that appeared after 30 days were microscopically tested for asci generation and then picked up on YEPG medium. The hybrid's resistance to selective antifungal drugs was identified to be tested for leavening ability [34]. The mated cells harboring the opposite genetic markers are presented in Table 4.

Table 4. Hybridization between yeast strains harboring the opposite genetic markers.

Parental genotypes	Hybrid genotype	Designation
(P_1) *Ben*$^+$ *Itr*$^-$ x (P_3) *Ben*$^-$ *Itr*$^+$	*Ben*$^+$ *Itr*$^+$	H_1
(P_1) *Gyn*$^+$ *Itr*$^-$ x (P_2) *Gyn*$^-$ *Itr*$^+$	*Gyn*$^+$ *Itr*$^+$	H_2

2.7. Preparation of dough

The dough was prepared from 325 g of wheat flour, 210 mL of yeast suspension in commercial tap water, 3.5 g salt, and 9.0 mL of sunflower oil, in addition to 0, 2, 4, and 8 g sugar. After adding all the ingredients, use the kneading arm for 15 minutes to manufacture the bread dough. Then the dough was placed in a 300 mL beaker containing 200 mL tap water and then incubated at 40 ^{0}C [35]. The time needed to ferment the dough was recorded according to [36].

2.8. Measurement of fermented bread dough weight

A direct assay of dough weight resulting from CO_2 generation was measured using a simple device method according to [37]. Immediately after the dough was prepared small pieces of dough were gently rounded, weighted, and placed inside the 300 mL baker each containing 200 mL tap water as a medium of fermentation, and then submerged in a water bath at 40 ^{0}C. The temperature of the water bath was adjusted to be constant at 40 ^{0}C. The fermentation activity was reported as the weight of bread dough in the baker. This increase in weight of bread dough during fermentation in the water medium was because carbon dioxide released by yeast cells was hydrated in the water into carbonic acid. The amount of gas produced was recorded as the increase in the weight of the bread dough piece after leavening time by subtracting the water absorbed by bread wheat at the end of fermentation time [38].

2.9. Leavening profile of dough

The dough mixture was poured into 300 mL measuring bakers each containing 200 mL tap water. After the rounded bread wheat was floated on the water surface, the fermentation time was recorded for each genotype [17].

2.10. Results expression

The experimental design included three trails. Values of parameters were expressed as the mean value according to [39].

2.11. Statistical analysis

The data were subjected to the analysis of variance (ANOVA) and the least significant difference (LSD) was used to compare between two means if the differences between treatments were significant at the P value > 0.05 [39]. The coefficient of variance reflected the homogeneity between hybrids with their parents was estimated as reported by [40]. Regression and correlation analysis were performed according to [40].

3. Results and discussion

3.1. Increase in fermented bread dough weight.

As shown from the results obtained in Table 5, the increase in fermented bread dough weight at zero concentration of sucrose was ranged between 0.14 (P_2) to 1.48 (P_3). Under the effect of supplementation with 2-gram sucrose, this increase was ranged between 0.14 (P_2) to 1.57 (P_3). The results of supplementation with 4 grams of sucrose showed that the increase in fermented bread dough weight ranged between 0.25 (P_1) to 1.97 (P_3). The ability of yeast to assimilate 6 grams of sucrose could increase the weight of fermented bread dough from 0.43 (P_2) to 1.67 (P_3). Meanwhile, the concentration of 8 grams of sucrose increased the weight of bread dough from 0.39 (P_2) to 1.46 (P_3). The results showed that hybrid H_2 produced heterosis in increasing the weight of bread dough over the mid parent which ranged from 46.81% at 6 grams of sucrose to 145.45% at zero supplementation of sucrose. Meanwhile, hybrid H_1 produced negative heterosis in increasing the weight of fermented bread dough if compared with the mid-parent. This indicated that this genotype was not able to increase fermented bread dough

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Table 5. Increase in fermented bread dough weight after fermentation under different sucrose concentrations.

Genotypes	Sucrose concentrations (g / 325 g wheat flour)				
	0	2	4	6	8
P_1	0.51	0.68	0.25	0.51	0.52
P_2	0.14	0.14	0.51	0.43	0.39
Mid-parent	0.33	0.41	0.38	0.47	0.46
H_2	0.81	0.67	0.77	0.69	0.75
Heterosis	145.45	63.41	102.63	46.81	63.04
P_1	0.51	0.68	0.25	0.51	0.52
P_3	1.48	1.57	1.97	1.67	1.46
Mid-parent	1.00	1.13	1.11	1.09	0.99
H_1	0.71	0.60	0.66	0.63	0.71
Heterosis	- 29.00	- 46.90	- 40.54	- 42.20	- 28.28
F – Test	*	*	NS	*	NS
LSD 0.05	0.75	0.65	1.31	0.82	0.83
0.01	1.09	0.94	1.90	1.20	1.20

NS = Not significant. * = Significance at 0.05 level of probability.

weight. These results indicated that hybrid H_2 exhibited bioactivity in the fermentation medium than hybrid H_1 at all sucrose concentrations. In addition, the parental strain (P_3) has the best genotypes than other parental strains used in this study for increasing the weight of bread dough during fermentation at all concentrations of sucrose. Therefore, hybrid H_2 resulted from hybridization between P_1x P_2, as according to the findings of this research well as, the parental strain P_3 is the best genotype for the bread dough-making industry. As a result, this is because of their higher bioactivity, high number of live - cells in fermentation medium, and high ability to increase the weight of bread dough than other yeast genotypes.

Therefore, more live yeast cells mean more viability and bioactivity which leads to more gas production power. For all genotypes used in this study, gas production was dependent on the yeast genotype which led to a high number of live cells because of more viability and bioactivity that lead to more gas production power. Higher quantities of gas production leading to high quantities of carbonic acid formed in the water medium, as a consequence the weight of fermented bread dough was increased. These results agreed with [38], who found a positive direct correlation between the survivability of yeast cells with gas production, power and the volume of bread. The same authors [38] reported that the high survivability and bioactivity of baker's yeast exhibited more gas production which leads to high height and volume in bread. Therefore, one of the most important discussions in dough fermentation is the survivability and bioactivity of baker's yeast cells. [41], decided that carbon dioxide released during dough fermentation is prominent as a leavening agent of dough. The assimilation of sucrose was varied among the yeast genotypes, as well as among the concentrations of sucrose which indicated some metabolic diversity that can be harnessed in industrial applications. It is interesting to note that supplementation of sucrose was not significantly affected on the production of carbon dioxide which is an important trait in bread making [42]. This indicated that the fermentable sugars present in the dough especially glucose and fructose are at enough concentrations to be converted into carbon dioxide which caused the doughs to rise and increase in their weight in water as a fermentation medium. Interestingly, the release of high quantifiable CO_2 by the parental strain P_3 and hybrid H_2 can be used in other processes where CO_2 gas can be trapped for commercial purposes. There are different sources of fermentable sugars in wheat flour, one of them is the sugars presented naturally in the flour including glucose, fructose, sucrose, and maltose, the second is any other fermentable sugars as sucrose added by the baker [43]. In sweet dough, the performance of yeast genotypes to ferment the sugars under high osmotic stress is of crucial and industrial importance [43]. High concentrations of sugar lead to high osmotic

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Table 6. Weight of fermented bread dough under osmotic stress.

Genotypes	Sucrose concentrations (g / 325 g wheat flour)				
	0	2	4	6	8
P_1	47.69	51.05	51.65	47.13	50.50
P_2	68.68	60.52	50.61	51.99	50.76
Mid-parent	58.19	55.79	51.13	49.56	50.63
H_2	47.67	50.33	54.41	49.97	55.88
Heterosis	- 18.08	- 9.79	6.41	0.83	10.37
P_1	47.69	51.05	51.65	47.13	50.50
P_3	50.21	50.54	53.57	50.83	47.97
Mid-parent	48.95	50.80	52.61	48.98	49.24
H_1	50.28	49.14	51.95	47.48	51.99
Heterosis	2.72	- 3.27	- 1.25	- 3.06	5.58
F – Test	**	NS	NS	NS	NS
LSD 0.05	10.06	10.59	15.82	12.21	13.71
0.01	14.63	15.40	23.02	17.77	19.94

NS = Not significant. ** = Significance at 0.01 level of probability.

stress because of the high concentration of alcohol released from high sugar concentrations. It is reported to be toxic to yeast cells due to the destruction of cell membranes which inhibits the growth of yeast cells [44].

An increase in the weight of fermented bread dough during the fermentation in the water medium may be due to carbon dioxide productivity, which can hydrate in the water into carbonic acid (H_2CO_3) and bicarbonate ions (HCO_3^-) leading the weight and volume of bread dough to be increased in the water as a medium of fermentation. The reaction scheme was written according to [45], as follows:

$$CO_2 + H_2O \underset{K_1'}{\overset{K_1}{\rightleftharpoons}} H_2CO_3 \underset{K_2'}{\overset{K_2}{\rightleftharpoons}} HCO_3^- + H^+ \rightleftharpoons CO_3^{2} + 2H^+$$

$$CO_2 + H_2O \longrightarrow H_2CO_3$$

$$H_2CO_3 + H_2O \longrightarrow HCO_3^- + H_3O^+$$

The highest concentration of H^+ was about 10^{-4} mol dm^{-3}. The reaction rates are mainly measured by the charge density at the carbonylic carbon atom. The collinear structure of CO_2 is unique among all other carbonyl compounds. Therefore, drinking water contains different concentrations of CO_2 which reflect their acidity [46]. Thus, the acidity of water refers to the transformation of CO_2 to carbonic acid. The acidity of water increased with increasing the productivity of CO_2, as well as CO_2 gas dissolution. This is in line with [47], who reported that the rate of CO_2 diffusion in absorbed water increases with increasing water temperature in the range from 50-75° C. Therefore, CO_2 molecules move faster in the fermentation medium of warm water used in this study, which results in higher sensitivity for the movements of CO_2 molecules through the water layers absorbed by fermented bread dough during the fermentation process in warm water. The gas cells present in the dough were expanded by the accumulation of carbon dioxide produced by baker's yeast. Carbon dioxide present in dough can be found as gas trapped inside the gas cells and dissolved in the aqueous phase leading the weight of fermented bread dough to be increased [24].

3.2. Bread dough weight

As shown from the results tabularized in Table 6, there were significant differences between genotypes concerning the weight of fermented bread dough without any supplementation of sucrose. In contrast, all concentrations of sucrose revealed insignificant differences between the different genotypes for the weight of bread dough. Hybrid$_2$ (H_2) appeared heterosis in the weight of fermented bread dough at 4, 6, and 8 g. This heterosis ranged between 0.83% at 6 g to 10.37% at 8 g. These results are due to the bioactivity of hybrid cells at the high concentrations of sucrose in the fermentation medium. Meanwhile, hybrid$_1$ (H_1) appeared positive heterosis reached to

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2.72% and 5.58% at zero and 8 g, respectively. Therefore, the hybrid genotypes differed in their bioactivity for increasing the weight of fermented bread dough during the fermentation time in water as a medium of fermentation.

These results indicated that H_2 exhibited high heterosis (10.37%) for the weight of fermented bread dough at the high concentration of sucrose (8 g) in the fermentation medium. Meanwhile, H_1 appeared to have the same trend at the same concentration of sucrose in the fermentation medium, when the heterosis reached 5.58%. The results reflected that H_2 and H_1 had more living cells at the higher concentration of sucrose. Therefore, yeast bioactivity had a direct correlation with the weight of fermented bread dough during fermentation time. So, more vitality of yeast hybrids leads to more bioactivity as a consequence of high quantities of gas production. Hybrid yeast cells had higher amounts and higher bioactivity of live cells which led to higher gas production power than the parental strains. According to these results, hybrid yeast cells were the best samples in the bread dough-making industry because of their technical terms of bioactivity, survivability, and high number of live cells at the high concentration of sucrose in the fermentation medium. This leads to a high ability in gas production that influenced to increase the weight and volume of bread fermented dough during the fermentation time. These results are in harmony with [38], who found that yeast A strain genotype had the highest number of green cells and the highest number of viable cells due to its bioactivity if compared with types B, C, and D genotypes which had the lowest number of viable cells. In gasography, [38] found that yeast strain A genotype produced the highest amount of CO_2 leading to the highest volume and height of bread dough, whereas yeast strain D genotype produced the lowest amount of CO_2, as well as the lowest volume and highest of fermented bread dough. For all yeast strains and their hybrids used in this study the weight of fermented bread dough after the fermentation time differed from one genotype to another. This is due to differences in gas production rate which affects the weight of bread dough over time. Therefore, more vitality of yeast cells as shown in hybrid cells used herein reflected bioactivity, gas production powering, as well as high weight, height volume, and height of bread dough. In the same criteria, [38] decided that the yeast had a higher amount of live cells and had higher gas production power than other yeasts. So, the hybrids generated in this study exhibited higher performance in the weight of bread dough than the parental genotypes, especially at the higher concentration of sucrose. More number of hybrid live cells means more survivability and bioactivity which leads to more gas productivity power which increases the weight of bread dough over time in water. So, the hybrid genotypes were superior in bread dough fermentation over time in a water medium for their survivability and gas production power leading to increase in the weight of fermented bread dough. These results agreed with [48], who demonstrated high levels of leavening activities in a total of 12 yeast genotypes when compared with commercial yeast. It is interesting to note that the high production of carbon dioxide by yeast hybrids is an important parameter for the commercial yeast-making industry. These hybrids were able to assimilate the high concentration of sucrose tested to be producing a high weight of fermented bread dough in relation to the mid-parent. Therefore, yeast specifically hybrid genotypes of *Saccharomyces cerevisiae* was preferred to be used in manufacturing bread dough as a leavening agent, where is better converts the fermentable sugars present in the dough into carbon dioxide which transfers in water medium to carbonic acid leading the weight of fermented bread dough to be increased. This causes the dough to expand and rise in weight because the carbon dioxide forms pockets or bubbles full of carbonic acid [49]. Bread dough manufacturing is supported by the abilities of yeast hybrid genotypes to ferment sugars present naturally in the dough especially glucose and fructose, in addition to the supplementation of higher sucrose concentration (8 g), the breakdown of which brings the release of carbon dioxide that caused the dough to rise in weight, volume, and height. The highest leavening activity recorded by hybrid genotypes indicates that it is the best biological wheat dough leavening agent obtained in this study because it can perform better fermentation than the parental genotypes used as controls. The results are in accordance with the findings of [50], who reported that species of

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Table 7. Estimated coefficient of variance for the increase in fermented bread dough weight under sucrose stress.

Genotypes	Sucrose concentrations (g / 325 g wheat flour)				
	0	2	4	6	8
P_1	0.021	0.121	0.153	0.096	0.039
P_2	0.048	0.123	0.159	0.114	0.459
Mid-parent	0.034	0.122	0.156	0.105	0.249
H_2	0.143	0.188	0.367	0.134	0.092
P_1	0.021	0.121	0.153	0.096	0.039
P_3	1.177	1.018	1.798	1.203	1.141
Mid-parent	0.599	0.570	0.975	0.650	0.590
H_1	0.222	0.125	0.280	0.094	0.046

Table 8. Estimated coefficient of variance for the actually weight of fermented bread dough under sucrose stress.

Genotypes	Sucrose concentrations (g / 325 g wheat flour)				
	0	2	4	6	8
P_1	0.076	0.059	0.065	0.132	0.037
P_2	0.202	0.039	0.026	0.156	0.061
Mid-parent	0.139	0.049	0.045	0.144	0.049
H_2	0.051	0.086	0.255	0.084	0.289
P_1	0.076	0.059	0.065	0.132	0.037
P_3	0.027	0.207	0.081	0.124	0.065
Mid-parent	0.052	0.133	0.073	0.128	0.051
H_1	0.021	0.033	0.198	0.187	0.076

Saccharomyces cerevisiae isolated from palm wine were observed to be the best genotypes in leavening wheat dough.

3.3. Degree of homogeneity

The estimated coefficient of variance values for increasing the weight of fermented bread dough in water as a medium of fermentation (Table 7) showed that hybrid$_2$ (H_2) recorded higher values at the following sucrose concentrations are 0, 2, 4, and 6 g than those of the mid-parent. This indicated the highest heterogeneity in the H_2 genotype since their cells recorded the highest coefficient of variance than the mid-parent. Then it could be considered as a new recombinant genotype. In contrast, hybrid$_1$ (H_1) reflected a coefficient of variance lower than those of the mid-parent, indicates that its genotype was enough homogenous with their parents.

Regarding the weight of fermented bread dough over the time of fermentation (Table 8), hybrid$_2$ (H_2) recorded a coefficient of variance higher than the check values of the mid-parent at sucrose concentrations are 2, 4, and 8 g. This leading H_2 could be considered heterogeneous, where its genotype induces high variation in the weight of fermented bread dough at most concentrations of sucrose. Meanwhile, hybrid$_1$ (H_1) showed the same trend of heterogeneity in the weight of fermented bread dough at high concentrations of sucrose including 4, 6, and 8 g. The degree of homogeneity was assessed depending on the coefficient of variability, which was used to determine the magnitude of diversity within every genotype, if compared with the check value in the mid – parent.

Therefore, both hybrids could be considered as new recombinant genotypes, since they exhibit a high coefficient of variance compared with those in the check value of the mid-parent. These results agreed with [51], who found that 16 new lines of tomato exhibited high homogeneity based on the estimated coefficient of variance. Meanwhile, the reports [52] found that the coefficient of variance differed among tomato genotypes for the same trait and from one trait to another in the same genotype. The same authors obtained a great diversity among 15 selected genotypes for all the studied traits. Generally, the degree of heterogeneity differed among the hybrid genotypes for the same trait and from one concentration of sucrose to another by the same

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hybrid genotype.

3.4. Correlation and regression analysis

3.4.1. Weight of fermented bread dough

Regression analysis describes the relationship between the dependent variable weight of fermented bread dough and the independent variable sucrose concentrations of the P_1 genotype (Fig. 1).

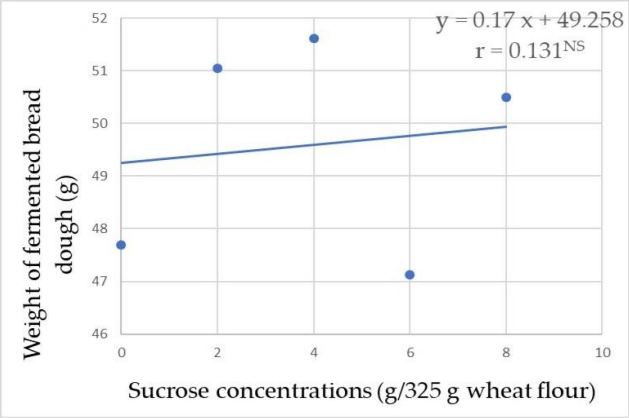

Figure 1. A scatter plot with the corresponding regression line and regression equation for the relationship between the response variable weight of fermented bread dough affected by the parental (P_1) genotype and the independent variable sucrose concentrations.

The regression line enables us to predict the weight of fermented bread dough from that of the independent variable sucrose concentration. Then, the regression coefficient (b = 0.17) represents the change in the dependent variable (weight of fermented bread dough) per unit of change in the independent variable (sucrose concentration). The regression coefficient requires attention to the units of measurement. The regression coefficient of 0.17 means that, in this model, a fermented bread dough weight increases by 0.17 g with each gram of sucrose added to the fermentation medium of the P_1 genotype. Therefore, the regression coefficient reflects the change in the dependent variable fermented bread dough weight that corresponds to a change in the independent variable sucrose concentration in the fermentation medium. The correlation coefficient in this relationship was equal to + 0.131. This means that $r^2 = 0.017$, therefore, 1.71% of the variance in fermented bread dough weight is due to sugar concentration. The remaining 98.28% could be attributed to other factors that were not taken into account in this analysis, such as the number of cells, survivability, bioactivity, etc. The positive correlation obtained herein indicates that the changes in both variables are in the same direction.

As shown in Fig. 2 concerning the P_2 genotype, the regression coefficient of - 4.437 means that the fermented bread dough weight decreases by - 4.437 g with each additional gram of sucrose. This may be due to the problem of sugar suppression leading to this genotype was sensitive to the higher concentration of sucrose. This agreed with [53], who reported that the acclimation of yeast genotypes to specific sugar as galactose reduced the glucose-induced repression on the transport of galactose, this repression leading to successful fermentation. The correlation coefficient (r = - 0.883) is a measure of how well the regression model describes the observed results. In this way, a negative association was obtained between the weights of fermented bread dough with sucrose concentrations. This indicated that if sucrose concentration increased, then the response variable bread dough weight decreased. Therefore, this relationship reflects that the changes in one variable are vice versa with the changes in the other variable. Such changes with the P_2 genotype are not in the same direction.

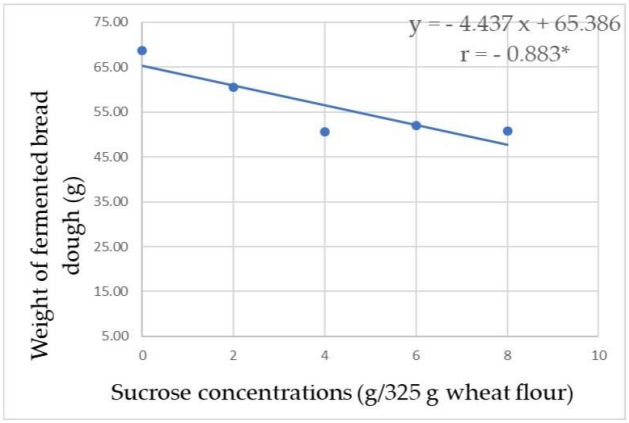

Figure 2. A scatter plot with the corresponding regression line and regression equation for the relationship between the response variable weight of fermented bread dough affected by the parental (P_2) genotype and the independent variable sucrose concentrations.

Fig. 3 demonstrated the relationship between the weight of fermented bread dough and sucrose fermentation by H_2 genotype, the regression coefficient of 1.606 means that fermented bread dough

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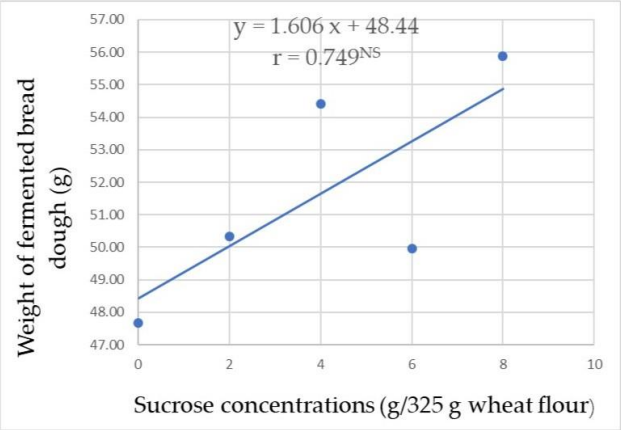

Figure 3. A scatter plot with the corresponding regression line and regression equation for the relationship between the response variable weight of fermented bread dough affected by hybrid$_2$ (H_2) resulted from the mating between P_1 x P_2 and the independent variable sucrose concentrations.

weight increases by 1.606 g with each additional gram of sucrose inside this model. Hybrid genotype H_2 showed a strong correlation (0.749) between the weight of fermented bread dough and sucrose concentrations. This indicated that if r = 0.749, then r^2 = 0.5610. This means that approximately 56% of the variance in the response variable can be explained by the explanatory variable. This agrees with the works of [54], who established that correlations above 0.7 are considered strong associations because if r = 0.7, therefore r^2 = 0.49. This reflects that approximately 50% of the variance in the dependent variable can be explained by the explanatory variable. The remaining 44% is due to other factors that were not taken into account in the analysis. These results indicated that the hybrid genotype H_2 was more tolerant to the high concentration of sucrose in the fermentation medium. These results agreed with [55], who decided that allele-specific expression in cis and in trans, is generally considered to play an important role in explaining the diversity between hybrids and parental lines. Therefore, heterosis is the phenomenon used to improve the performance of the progeny resulting from a cross between P_1 x P_2. Many of the improved traits are directly related to cell physiology and adaptation to the high concentration of sucrose. Thus, heterozygous genotypes perform better phenomenon in their gene expression in variable environments. Therefore, heterosis may lead to the formation of new species, because of increased genetic heterogeneity [55].

As shown from the results presented in Fig. 4, the regression coefficient between fermented bread dough weight affected by the parental strain P_3 and sucrose concentrations was equal to - 0.419. This means that fermented bread dough weight decreased by - 0.419 g with each additional unit gram of sucrose. These results indicated that the genotype P_3 was sugar-sensitive to the high concentrations of sucrose. These results agree with the findings of [56], who found that the addition of glucose inhibits the transcription of glucose-repressible genes. The correlation coefficient (- 0.332) describes the observed data, which indicated that the relationship between both variables was negative. Therefore, if the independent variable sucrose concentrations increased, then the response variable weight of fermented bread dough was decreased because the genotype P_3 was not tolerant to the high concentrations of sucrose. The line of regression does not closely approximate all the points in this Figure (Fig. 4).

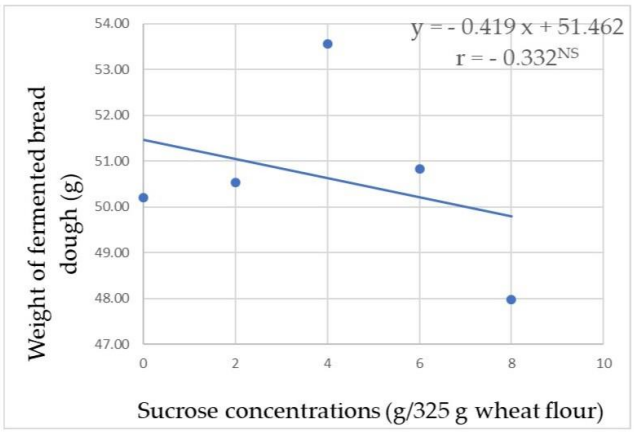

Figure 4. A scatter plot with the corresponding regression line and regression equation for the relationship between the response variable weight of fermented bread dough affected by the parental (P_3) genotype and the independent variable sucrose concentrations.

Rearrange Fig. 5 showed that the hybrid genotype (H_1) appeared to have regression coefficient of 0.176 in this model. This means that the weight of fermented bread dough increases by 0.176 g with each additional gram of sucrose inside this model. Therefore, the regression coefficient should be considered together with the units of all of the involved variables. A positive

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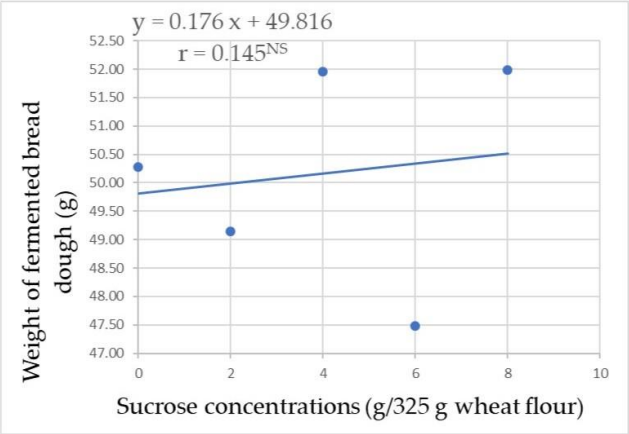

Figure 5. A scatter plot with the corresponding regression line and regression equation for the relationship between the response variable weight of fermented bread dough affected by hybrid$_1$ genotype (H_1) resulted from the mating between P_1 x P_3 and the independent variable sucrose concentrations.

association obtained between both variables indicated that the changes of these variables are in the same direction because the hybrid genotype H_1 was tolerant to sucrose concentrations. These results agreed with [57], who reported that in diploid species of yeast, the genetic loci showed additive, dominant, and epistatic effects. Therefore, the additive loci play a major important role in most traits. These loci produce the heritable trait variation in genetically diverse populations. *Saccharomyces cerevisiae* is a potential system powerful for studying non-additive genetic effects in diploids [57]. The results indicated that hybrids have no decrease in fitness, as expected if evolution was highly parallel if the divergent allele is compatible, or if heterozygosity is favored by selection. In addition, the hybrids exhibit breakdown in the trait if the opposite ancestry alleles are incompatible [57]. Theoretical studies [58] using geometric model of adaptation predict that hybrid breakdown is proportional to the magnitude of evolutionary change in parallel with the common ancestor [59]. Thus, the magnitude of heterosis as seen in this study would be greater in crosses between populations of the more derived benthic ecotype if compared with the crosses between limnetic populations, which are more ancestor-similar [60]. The reduction in the fitness of hybrids was caused by the segregation of incompatible alleles in recombinant hybrids which is termed hybrid breakdown [59]. However, the correlation coefficient between both variables is 0.145. This means that $r^2 = 0.0210$, therefore, 2.10% of the variance in fermented bread dough weight is due to sucrose concentrations. The remaining 85.4% is due to the genotype variation and other factors that were not taken into account in this analysis. These results agreed with [61], who demonstrated that if the relationship (r^2) between height and weight in humans is 0.785, this means that 78.5% of the variance in weight is due to the height and the remaining variance of 21.5% is due to the individual variation, which may be due to other factors that are not taken into account of the analysis such as age, exercise, sex, or eating habits.

3.4.2. Increase in fermented bread dough weight

The results diagrammatic in Fig. 6 demonstrated that the parental strain P_1 appeared to have a negative association between the increase in fermented bread dough weight and sucrose concentrations.

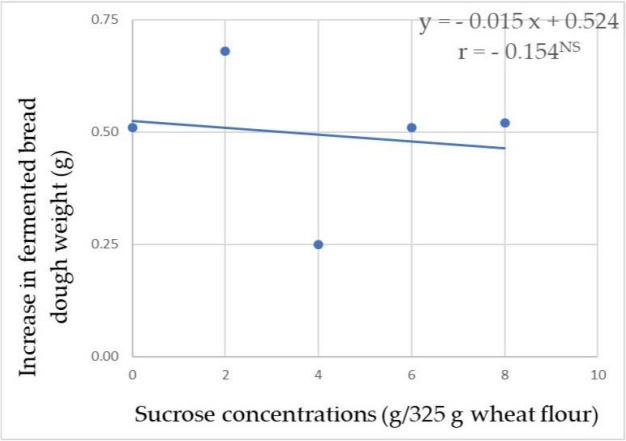

Figure 6. A scatter plot with the corresponding regression line and regression equation for the relationship between the response variable increase in bread dough weight affected by the parental (P_1) genotype and the independent variable sucrose concentrations.

The regression coefficient of this relationship of - 0.015 means that the weight of fermented bread dough decreases by - 0.015 g with each additional unit (g) of sucrose. The correlation coefficient (- 0.154) in this relationship describes the observed data. This means that the changes of both variables are not in the same direction because the genotype P_2 was intolerant to increasing sucrose concentrations.

The regression model in Fig. 7 revealed that the

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regression coefficient induced by the parental genotype (P_2) is 0.079. This means that, in this model, fermented bread dough weight increased by 0.079 g with each additional unit (g) of sucrose concentrations inside the figure. Thus, the correlation coefficient (0.728) describes the observed data. This reflected that r^2 = 0.5299, therefore, 52.99% of the variance in increasing fermented bread dough weight is due to sucrose concentration. The remaining 47.01% is due to other factors that were not taken into account in the analysis.

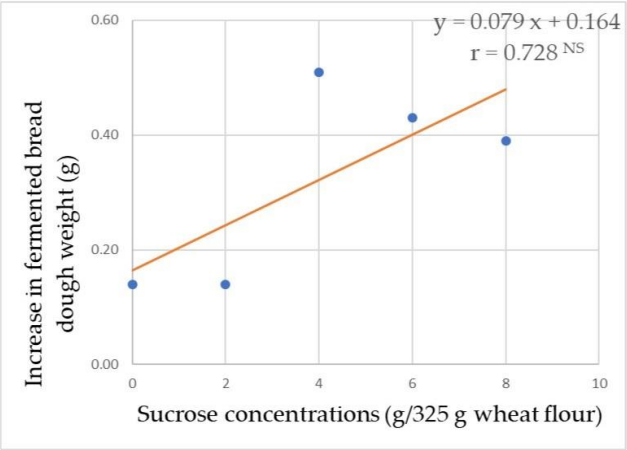

Figure 7. A scatter plot with the corresponding regression line and regression equation for the relationship between the response variable increase in bread dough weight affected by the parental (P_2) genotype and the independent variable sucrose concentrations.

These results indicated that the P_2 genotype was tolerant to sucrose concentrations used in this model. These results are in harmony with [62], who reported that heterosis confers a competitive advantage by facilitating transgressive phenotypes in changing environmental conditions, which is a driver in fungal evolution and adaptation. This is important for yeast cells as many stages of fermentation and maturation develop a microbiologically competitive environment in which quick adaptation may be advantageous. Therefore, interspecific hybridization is a valuable technique for yeast development, enabling the combination and enhancement of the traits from both parental strains or species [63]. The hybrid development technique in this study is the rare mating of spores. This approach bears a highly successful, high-stability genome. These results in yeast influencing gene dosage during cellular processes which explains the outperformance over a diploid yeast of the same genetic background [63]. The rare mating spores needed selection markers to perform this technique, it is estimated to occur in one out of 10 million cells [64]. In a rich nutrient environment of yeast, mother cells reproduce asexually to form daughter clones. Under poor conditions of nitrogen as proline, the growth of yeast was changed to form a pseudohyphal [65]. Under the complete absence of nitrogen with the presence of a non-fermentable carbon source such as acetate, the yeast cells are sporulated [66]. The cell wall was transformed into the ascus during sporulation which holds four spores named tetrad. These spores divide equally into mating types as either a or α [67]. If the conditions improve, new haploid yeast (1n) can conjugate with the opposite mating type as they form a shmoo. Heterosis is often found during the early stages of divergence, followed by declines over longer time spans, as seen in the parental strains P_1 and P_2 genotypes when hybrid breakdown is enduring [68].

As shown from the results presented in Fig. 8, the regression coefficient between the increase in fermented bread dough weight affected by the parental genotype P_3 and sucrose concentrations was equal to 0.006.

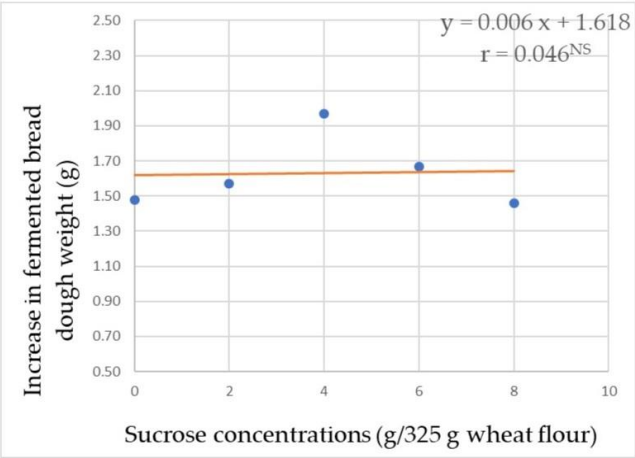

Figure 8. A scatter plot with the corresponding regression line and regression equation for the relationship between the response variable increase in bread dough weight affected by the parental (P_3) genotype and the independent variable sucrose concentrations.

In this model, this means that the weight of fermented bread dough increases by 0.006 g with each additional unit (g) of sucrose. The correlation coefficient between

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both variables describe the observed data. The correlation coefficient in this case is 0.046. This means that r^2 = 0.002, therefore 0.2 % of the variance in increasing fermented bread dough weight is due to sucrose concentrations. The remaining 99.80 % may be due to other factors that were not taken into account in the analysis.

According to Fig. 9 which describes the relationship between the increase in fermented bread dough weight affected by the hybrid H_1 genotype and sucrose concentrations, the regression coefficient is 0.003. This means that fermented bread dough weight increases by 0.003 g with each additional unit (g) of sucrose. The correlation coefficient between both variables is 0.097. This means that r^2 = 0.0094, therefore, 0.94% of the variance in increasing fermented bread dough weight is due to sucrose concentrations. The remaining 99.06% is due to other factors that were not taken into account in the analysis. Therefore, the association between both variables was in the same direction.

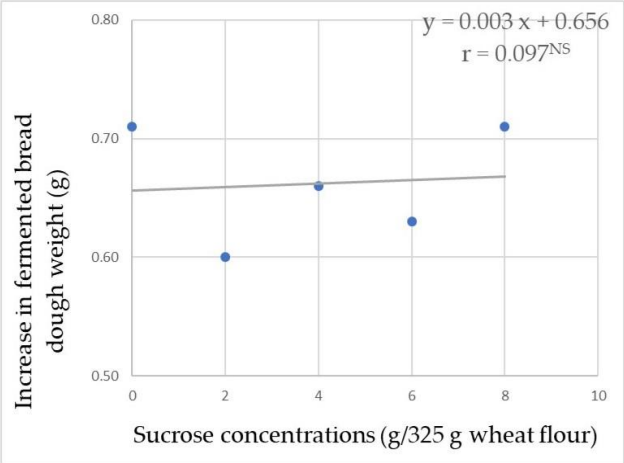

Figure 9. A scatter plot with the corresponding regression line and regression equation for the relationship between the response variable increase in bread dough weight affected by hybrid genotype (H_1) resulted from the mating between P_1 x P_3 and the independent variable sucrose concentrations.

As shown in Fig. 10 concerning the relationship between the response variable increasing fermented bread dough weight and the independent variable sucrose concentrations, the hybrid genotype H_2 appeared regression coefficient of - 0.01. This means that bread dough weight decreases by - 0.01 g with each additional unit of sucrose (g). The correlation

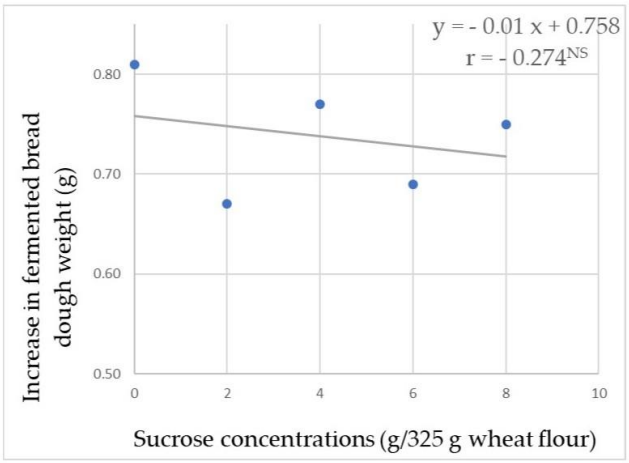

Figure 10. A scatter plot with the corresponding regression line and regression equation for the relationship between the response variable increase in bread dough weight affected by hybrid genotype (H_2) resulted from the mating between P_1 x P_2 and the independent variable sucrose concentrations.

coefficient describes the observed data which equals - 0.274. This means that r^2 = 0.0750, then 7.50% of the variance in increasing the weight of fermented bread dough was due to sucrose concentrations and the remaining variance of 92.5% is due to other factors that were not taken into account in the analysis as yeast genotypes, fermentation time, dough ingredients, temperature ect. The negative value of association between both variables indicated that the changes in both variables are not in the same direction. This means that bread dough weight was decreased with each unit increase in sucrose concentration. This indicated that the genotype H_2 was intolerant to sucrose concentrations. These results showed that the hybrid genotypes showed better expression in two *FDH* genes (FDH_1 and FDH_2), their encodes converting weak acids into carbon dioxide [69]. Therefore, the hybrid exhibited intermediate properties in relation to the parental strains. The combination of positive alleles in hybrid genotypes leads to final superiority by the effect of dominance [70] or over dominance [71]. This is in harmony with [72], who found that some progenies of yeast clones reached about 13% revealing a good trait value as the best parent. This may be due to the enhancer loci which are located in the better parent and the silencer loci in the other one [73]. In addition, trans-aldolase (*TAL*) genes and format dehydrogenase (*FDH*) genes encoded conferred resistance to weak acids in the recombinant sucrose-fermenting yeast [74]. Therefore, [69] decided that *FDH* genes in recombinant hybrids of yeast may be regulated in the hybrids of

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Saccharomyces cerevisiae. The hybrid yeast genotypes showed better adaptation to stress conditions of sucrose in the fermentation medium than parental strains because the genes related to the bioconversion of sucrose into ethanol, carbon dioxide, and other secondary metabolites may be naturally expressed, therefore, they must be preferred in industrial applications. This agreed with [75], who found that hybrid yeast genotypes were able to resist higher glucose concentrations in grape musts than the parental strains. Thus, the genome recombination in yeast released a novel population of hybrid genotypes with further improvements in fermentation, which illuminated the significance of heterosis in the improvement of fermentation in industrial yeasts. This is because the hybrid genotypes induced superior gene expression of fermentation and adaptation genes than those of either parent. Therefore, hybrid genotypes showed better acclimatization to the high concentration of sucrose to reduced sugar repression, which affects the transport of sugars as galactose. This is because sugar repression had to overcome the successful fermentation [53]. It is essential to show the output of heterozygosity that mitochondrial gene loci undergo recombination in heteroblastic cells [76]. In this criteria, [77] demonstrated that diploid yeast strains repressed or depressed to glucose contain the same amount of mitochondrial DNA packaged in approximately 4-5 mitochondrions in repressed cells or about 22 mitochondria in derepressed cells. Therefore, mitochondrial DNA is another significant factor affecting sugar repression [78] decided that one mole of glucose in the fermentation medium was converted into two mol of ethanol and two mol of carbon dioxide. Thus, the ability of yeast genotypes to produce ethanol and carbon dioxide depends on the initial concentration of sugars in the fermentation medium. Increasing sugar concentrations in the fermentation medium led to increasing osmotic pressure as appeared a negative effect on yeast genotypes, as shown by the parental strains used in this study. Therefore, the yeast genotypes need to adapt to changes in their fermentation medium to survive. Thus, [79] decided that *Saccharomyces cerevisiae* is an acidophilic microbe that grows better under acidic conditions ranging from 4 to 6. Furthermore, ethanol and CO_2 are the major fermentation products of glucose, fructose, and sucrose. This is in line with [80], who found that produced ethanol was apparently more toxic for yeast genotypes than added ethanol. [81] decided that hexokinase II may enter the nucleus to regulate the repression of at least the *SUC*$_2$ gene by glucose. The phosphorylation of hexokinase II was necessary to enter the nucleus and initiate glucose repression [81].

4. Conclusions

The quest to gain diverse genotypes in baker's yeast populations and novel fermentation characteristics from the parental genotypes remains an overarching goal for microbial geneticists. Hybridization represents an important source of genomic and phenotypic diversity, which plays a significant role in the evolution of yeast strains. The leavening ability of dough during bread production is a result of carbon dioxide produced by *Saccharomyces cerevisiae*. Yeast converts the fermentable sugars present in the dough into carbon dioxide. This caused the dough to expand as a result of carbon dioxide forming bubbles in the bread dough, giving the product a soft and spongy texture. Regression analysis is a powerful technique with many implications for microbial genetic research. It enables microbial geneticists to describe, predict, and estimate the relationships between the interrelated variables when the researchers are interested in examining the relationship among specific variables. The results obtained in this study detected a positive correlation between the weight of fermented bread dough that was leavened by P_1, H_1, and H_2 genotypes and sugar concentrations. The weight of fermented bread dough was increased in the water medium of fermentation. This refers to the transformation of carbon dioxide into carbonic acid which leads to fermented bread dough to increase their weight and decrease their density. In contrast, if the fermentation medium is the atmosphere instead of water then the weight of fermented bread dough, it decreases due to CO_2 production. This causes the dough volume to expand and reduced in its weight and density. Therefore, the decrease in dough density in the water medium of fermentation leads the dough cores to floating on the water surface. Hybrid genotypes exhibited better fermentation activity than their parents. The leavening value of hybrid yeast cells was due to their viability and increasing in their numbers under sucrose stress. This drove the hybrid genotypes to produce more quantities of CO_2, leading the dough volume to expand and reduce in density.

Ethical consent

This study does not indicate any human or animal

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testing or feeding on irradiated products.

Authors' contributions

It is not applicable because a single author constructed this manuscript.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The author declares that this manuscript was done in the absence of any commercial or financial relationships that could be conducted as a potential conflict of interest.

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