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Comparative Evaluation of the Dynamics of Alcohol Producton of Wine Yeast Strains Isolated in Tokaj Region

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Abstract- The dynamics of ethanol production of wine yeasts were examined in model experiments as well as in the winery. The ethanol concentration in young wines fermented by local strains of *Saccharomyces cerevisiae*, *S. uvarum* or *Starmerella bacillaris* (21, 2 and 2, respectively) did not vary considerably (c.v. 1.9 %). All of them produced significantly higher amount of ethanol than the type strain [ATCC 26108] of *S. cerevisiae*. However, their performance during the fermentation process diverged significantly. Thus the lag phase varied between 33 and 123 hours, while the time requested to produce half of the final ethanol concentration varied between 67 and 294 hours.

Keywords: yeast, wine, fermentation dynamics, saccharomyces, starmerella, tokaj.

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Comparative Evaluation of the Dynamics of Alcohol Producton of Wine Yeast Strains Isolated in Tokaj Region

Zoltán Kállai [α], Zsuzsa Antunovics [σ] & Gyula Oros [ρ]

Absract- The dynamics of ethanol production of wine yeasts were examined in model experiments as well as in the winery. The ethanol concentration in young wines fermented by local strains of *Saccharomyces cerevisiae*, *S. uvarum* or *Starmerella bacillaris* (21, 2 and 2, respectively) did not vary considerably (c.v. 1.9 %). All of them produced significantly higher amount of ethanol than the type strain [ATCC 26108] of *S. cerevisiae*. However, their performance during the fermentation process diverged significantly. Thus the lag phase varied between 33 and 123 hours, while the time requested to produce half of the final ethanol concentration varied between 67 and 294 hours.

The dynamics of ethanol production differed at high degree between *S. cerevisiae* strains isolated of several vintages of local wines (c.v. 25 %), where the intensity of specific ethanol production (ISEP) varied between 0.81-4.56 % ethanol per day. Reverse relationship was revealed between the Lag phase and the ISEP (r^2=0.858, $p>0.01$), and the circumstances of fermentation did affect this trend. Based on their properties, *S. uvarum* and *St. bacillaris* strains applied nowadays in wine making have been positioned in the ranges of *S. cerevisiae* strains.

Baule-Mitherlich, Gompertz, hyperbolic, logistic, logarithmic, polynomial, and probit functions were applied to analyze the dynamics of fermentation. All functions fitted well to experimentally measured values at the range of 2 to 9 % of ethanol, that means, the half time could be approached by any of them at $p<0.05$ level. However, the predictive power of these functions differed significantly; both Lag phase and End point of fermentation could be calculated with requested precision ($p<0.001$) only with a polynomial function. The constant and secondary coefficients of this function counteracted to the primary one strictly in strain dependent manner, and the role of these three factors groups also varied in strain-dependent manners during the vinification process.

Keywords: yeast, wine, fermentation dynamics, saccharomyces, starmerella, tokaj.

I. Introduction

The wine is an alcoholic drink usually fermented from grape juice by yeasts, and it is the result of the transformation of sugars into ethanol and carbon dioxide. This process has been well studied since the pioneer works of Pasteur, and numerous papers have focused on the dynamics of yeasts during the wine fermentation elucidating the role of *Saccharomyces* species. Among them, *S. cerevisiae* is considered to be primarily responsible for ethanol production metabolizing sugars *via* the fermentative pathway when the sugar concentration is high, and this species is widely preferred for initiating fermentation.

More than 40 of the 1500 known yeast species were isolated from grape must [1]. Nevertheless, some species of diverse microbiota presented in the vineyards [2, 3] and musts [4, 5] are also involved into the fermentation during the first stages of winemaking [6-9]. Still, studies comparing yeast ecologies in vineyards and cellars clearly showed that the yeasts present on grapes are subject to natural phenomenons as grape maturity and weather, as well as to human interventions and the phytosanitary treatments carried out [10, 11]. Thus, in oenological conditions, these species due to their low capacity to multiply and their particular needs for micronutrients and oxygen [12, 13] have limited fermentation capacities compared to *Saccharomyces* yeasts, which are adaptable to hostile conditions[14]. Consequently, the populations of residual indigenous yeasts quickly decrease [15], and most of them disappear when the ethanol concentration increases over 4–5% (v/v) [16].

Nowadays, *S. cerevisiae* and *S. uvarum* are the leading species of alcohol fermentation. Still, *St. bacillaris* and *Torulaspora delbrueckii* also able to complete the alcohol fermentation [17], and these yeast species became a concern of interest in modern winemaking. Moreover, due to the consumer oriented wine markets, there is an ever-growing quest for specialized wine yeast strains possessing a wide range of optimized, improved or novel oenological properties [18], and winemakers have started to believe in the synergetic effect of some non-*Saccharomyces* species in matters such as aroma intensity and complexity [19] (Table 1), as the incidences of non-selected

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Saccharomyces or non-*Saccharomyces* opportunistic yeasts during fermentations were usually related to off flavors improving the overall quality of the wines [20, 21]. Nevertheless, their secreted enzymes could be detected throughout the fermentation process [22, 23], impacting the wine fruitiness and complexity [24].

In present times, due to fears towards GMO technologies and legal regulations, researchers have turned their attention to the *Saccharomyces sensu stricto* group. It is well known that members of this group can hybridize with each other in the nature. Under laboratory conditions any of the *Saccharomyces* species can form hybrids with any other species of the genus [25-43]. Hybridization brings all alleles of all relevant genes of different strains together and recombines them during segregation/chimerization of the hybrid genomes. The hybrids and their chimeric derivatives can outperform the parental strains in technologically relevant properties including stress response [41]. There are countless possibilities in this mechanism that can be exploited to design and create strains optimized for industrial tasks. For instance, comparative genomic analyses revealed that the thiol-releasing wine yeast, VIN7, has an allotriploid hybrid genome with *S. cerevisiae* and *S. kudriavzevii* origins [44] that explained the genetic basis of this VIN7's unique capacity to produce wines with a distinctive guava-like aroma [18].

By adopting and authorizing GMO technologies, the creation of engineered industrial strains can be accelerated, and strains optimized for a given task, expected aroma production, or even specific vintage conditions can be created. With the confluence of modern-day biomolecular sciences, information technology, and engineering, the DNA of yeasts can now be redesigned, reinvented, rewritten, and edited with astounding precision [45-48]. Engineering the biology of a model and non-model yeast strains (including clonal variants of natural isolates, mutants, hybrids, and genetically-engineered GM strains) with laser-sharp accuracy can stretch the realms of possibility in yeast research and wine yeast innovation [49]. Some attempts have already come to light; for example, the haploid wine strain (AWRI1631) of *S. cerevisiae* was equipped with a biosynthetic pathway, which consists of four separate enzymatic activities required for the production of the raspberry ketone [50].

Over a thousand papers have been published on the use of sequential yeast mixtures in wine fermentation. The aim of these efforts was to regulate the vinification process as well as to direct the ethanol production from organic acids [14, 51-53]. Moreover, much interest has been developed to the low alcohol content and in the use of different wine yeast species to improve sensory impacts of vine grape varieties for wine utilizing the aromatic potential of some non-*Saccharomyces* yeasts [54]. The population dynamics of various strains alone or in mixtures as well as the kinetics of sugar consumption and carbon dioxide production have been examined in details with diverse methods, and selection of appropriate parameters of kinetics for comparative studies has been discussed [55-60]. We focused our attention on the kinetics of alcohol production with regard to varietal differences in the time course of the process. In the present study, we performed fermentations in laboratory models and semi-industrial scale to compare *St. bacillaris* and *S. uvarum* strains to *S. cerevisiae*, all isolated from the Tokaj (Hungary) region. The experimental data were analyzed with Baule-Mithrelich, Gompertz, hyperboloid, logistic, logarithmic, polynomial, and probit functions, and manual fitting in Descartes plots to reveal the usefulness of kinetic parameters of alcohol production in comparative studies.

II. Materials and Methods

Data on maintenance, origin, and methods of authentication of wine yeast strains used in model experiments (Table 2) were reported in detail by Kállai et al. [61].

Microvinification: 50 mL autoclaved Yellow Muscat must (204.3 gL^{-1} sugar, pH 3.38) prepared from grapes harvested in Tarcal was inoculated with cells of an overnight culture to obtain $5x10^6$ cell mL^{-1} concentration and it was incubated at 12°C without shaking for 30 days. The tests were carried out in two series.

Semi-industrial fermentation in the winery: The Furmint grape must (204.3 gL^{-1} sugar, pH 3.2) was cleaned with a vacuum drum filter and equalized. The inoculation concentration was 5×10^6 cells/ml of the must. The fermentations were carried out in 100 L steel tanks for 30 days. Samples were taken by time course given in Figure 1 to observe the dynamics of the fermentation.

Analytics: The alcohol, glucose, fructose, total sugar, and acetic acid concentration was measured with a Bruker Alpha FTIR spectrometer (Bruker Optic GmbH, Germany) and the results were processed with the Bruker OPUS software.

Data analysis: Fisher's test was applied to evaluate the significance of differences between variants at $p = 0.05$ level. The average values of ethanol concentrations determined in samples were used to construct two data matrices; the first comprised data of *S. cerevisiae* strains fermenting Yellow Muscat juice (20×12; strain×time), while data of strains of three species (*S. cerevisiae*, *S. uvarum* and *St. bacillaris*) fermenting Furmint juice ($3\times2\times12$; species×strain×time) were put into the second one.

Both data matrices comprising time-dependent percentage values were subsequently analyzed by percent of ethanol *versus* log time regression applying Baule-Mitherlich, Gompertz, hyperbolic, logistic,

logarithmic, polynomial and probit functions to elucidate the character of dynamic changes in ethanol production during the fermentation following models described by Sváb [62]. The kinetic parameters (Lag phase, Half time of alcohol production, and End point) were also extrapolated by manual fitting on Descartes's plot, and the values calculated by the above functions were correlated to these values by linear regression. Box & Whiskers plots were used to demonstrate differences both in the fitness of regression and predictive power of equations to evaluate the applicability of the examined regression models.

Statistical functions of Microsoft Office Excel 2003 (Microsoft, Redmondton, USA) and Statistica5 programs (StatSoft, Tusla, USA) were used for multivariate analysis of data. Graphical presentations of the results of data analysis were edited uniformly in MS Office PowerPoint 2003.

III. Results and Discussion

The levels of ethanol in the medium were determined with high accuracy ($F_{repl}=1.37 > F_{0.1}=2.88$). The ethanol production of strains was different and varied between 11.65 and 12.95 % v/v ($F_{Strain}=2.281 > F_{0,05}=2.11$; $p<0.05$). All strains isolated in the Tokaj region [61] performed better than the type strain (ATCC 26108) of *S. cerevisiae* (Table 2).

Plotting the actual ethanol concentrations determined analytically *versus* time of sampling (Fig. 1) revealed significant strain-dependent dynamics of ethanol accumulation ($F_{exp}=18.2 > F_{0.001}=15.38$) during the vinification process, where four periods could be significantly distinguished ($F_{Time}=1532.9$, $p<0.001$) in each case. Thus, the detectable amount of ethanol got out after lag phase (P_1) succeeded first with rapid then descending increase (P_2 and P_3), and the process terminated with slow changes (P_4) up to the ethanol level characteristic to the strain concerned. The length of these stages can be determined manually, plotting the experimental data. This easy to handle method allows the assessment of the character of changes of alcohol concentration *versus* time as well as the crude approach of several kinetic parameters (Lag phase, Half time, the Specific rate of alcohol production). However, the fitness of correlation can not be precisely evaluated. The high variations in measured values both in parallel batches and performance of strains during the process (Fig. 2) indicate changes in the roles of influencing factors in vinification process of grape juice, first of all in the start of ethanol production (period P_1), but parallel to the increase of ethanol concentration (over 5 % v/v) this variation decreases. Its varietal difference rapidly diminishes (c.v. < 1%). Analyzing the time-dependent changes in ethanol concentrations on manually fitted scatterplot (Fig. 1) the use of sigmoid (logistic and Gompertz functions) and saturation (Baule-Mitscherlich, hyperbolic and logarithmic functions) models seemed to be plausible. Moreover, the applicability of the square approach (polynomial function) and linearization *via* probit transformation were also tested.

Comparing the fitness of various approaches, the sigmoid type models proved to be applicable to our set of data with limitations, because of the strong asymmetry of the fermentation dynamics (Figs. 1 and 2). Although, both logistic [63, 64] and Gompertz [56, 65, 66] functions were proposed for the analysis of dynamics of must fermentation, in our case these seem to be useful with care, because of the lack of data at the start of the process (stages P_1 and P_2 in Fig. 1). Thus some related results of calculations have been omitted of the comparative analysis of models. The determination coefficients showed high and strain-dependent variability of the fitness of regressions (Fig. 3) in the case of both logistic (symmetric sigmoid, $r^2=0.74$-0.99) and Gompertz (asymmetric sigmoid, $r^2=0.70$-0.99) functions. Due to extremely high variation after inoculation of grape juice, - as it was mentioned above, - we assume that the more frequent sampling in this period could not improve the exactitude of the extrapolations based on the sigmoid functions.

The analysis of a manually fitted scatter plot (Fig. 1) corroborates the suggestion of the use of saturation models as well. Meanwhile, both logarithmic and hyperbolic functions can be directly applied using experimental data, the Baule-Mitscherlich (BM) model, like the sigmoid one, requests a limit that can be determined by either iteration or giving a fixed value. In our case the iterated limit of BM function resulted in irrationally high ethanol concentrations (14-17 v/v % depending on the strain concerned). Thus we fixed the limit of this model in maximum ethanol concentration measured analytically in new wine produced by the actual strain. The determination coefficients in the case of BM model varied in strain-dependent manner ($r^2=0.85$-0.98) but to a lesser extent than in the case of sigmoid functions (see Fig. 3). The fitness of logarithmic regression varied in strain-dependent manner ($r^2=0.87$-0.99) at a lesser extent than BM one. The hyperbolic function proved to be much better ($r^2=0.94$-0.99). However, the median was less than $r^2=0.98$ in each saturation model.

The linear relationship was also used for studies the kinetics of fermentation [67]. Still, in our case the linearization of experimental data with probit function did not improve the fitness of time-dependent regression as compared to other models (Fig. 3), the variation of determination coefficient varied within wide limits ($r^2=0.84$-0.99) in this case too.

The second-order polynomial function proposed by several authors [68, 69] was applied to test the square approach. This model surpassed all others involved; the determination coefficients were over 0.95 in each case, and the median was over 0.99, which means

this function showed less strain-dependent variation in fitting the regression than other models tested (Fig. 3). The result of calculations based on the square approach was demonstrated in Figure 4 using strains of *S. cerevisiae* and *St. bacillaris*. The calculated curves fit excellently to analytically determined values of alcohol concentration of the start to the endpoint of fermentation. The expected alcohol content of new wines extrapolated applying polynomial function fitted well to the analytically measured values, contrary to the other models, where the extrapolations resulted in high and strain-dependent alterations (Fig. 5). Thus, this model was applied for calculation of specific ethanol production (Table 2), which intensity varied between 0.81-4.56 % EtOH per day (0.797-1.396 mM per hour). All local strains surpassed the type strain [ATCC 26108], which produced 0.81% ethanol per day (0.517 mM per hour). Reverse relationship was revealed between the Lag phase and the ISEP (r^2=0.81, $p>0.01$), and the circumstances of fermentation did not affect this trend. No relationship could be elucidated among other known properties of strains and intensity of their specific ethanol production rate. As it was demonstrated in Figure 6, the ISEP is not connected to oenological properties of strains, and can not be linked to their taxonomic position either (Fig. 7). The other kinetic parameters, calculated applying various functions, showed large variation in strain-dependent manner as well (Fig. 8). The continuance of both lag phase and half time extrapolated using polynomial function were more similar to experimental values than those computed by any other functions (Table 3).

The polynomial function permits to weigh the role of constant, primary, and secondary effects as well as to analyze their relationships in strain-dependent manner (Table 4). The actual ethanol concentration (Y) is a product of working cell factories and might be extrapolated applying polynomial function ($Y=A+b_1\times[X]+b_2\times[X^2]$), where [X] is the actual time counted of the start of fermentation. At the same time, we can conceptualize the coefficients [A], [b_1] and [b_2] as vectors, i.e., sums of various factors influencing the ethanol-producing capacity of yeast cells in the vinification process. The [A] is a time-independent constant, which might be related to a group of properties of yeast strains that take part in ethanol production in a time-independent manners as well as not related to responses of cells to the changing environment in the fermentation tank. The influence of both [b_1] and [b_2] manifests in time-dependent mode, and can be considered to be vectors of primary and secondary factor groups, respectively, and these factors most probably take part in the regulation of the responses of cells to changes in environmental conditions. Our set of data allowed us to weigh their role in the regulation of dynamics of alcohol production. Their relationships in regulating the strain-dependent ethanol production during the time course of the vinification process shown in Figure 9. Surprisingly, strict trends ($p<0.01$) were elucidated in the manifestation of the simultaneous regulatory effect of these groups of factors. The [b_1] group of factors counteracted to both constant [A] and [b_2] groups. Moreover, the influence of factors [b_1] and [b_2] counteracting synchronously in time dependent manner was about two times stronger than the constant ones, meanwhile, the strength of [b_1] group surpasses that of the [b_2] one about five times. The yeast strains fit precisely to trend lines independently on their taxonomic position or other known properties. The weight of these factor groups changes during the vinification in a strain-dependent manner (Table 4). None of them dominated either P_1 or P_4 stages. The constant and secondary factors [A and b_2] counteracted to EtOH production in the second phase (P_2) contrary to primary one [b_1], and the strain-dependent ISEP negatively correlated to the effect of the primary factor group.

The changes of sugar and acid levels in vinification batches of our strains were checked over during fermentation earlier [61], and these data were used for the multiple regression analysis (Table 5). The strain-dependent glucose utilization was connected to strain-dependent dynamics of alcohol production only in the start (period P_1) then the differences between strains in this respect became negligible. Meanwhile, the strain-dependent intensity of fructose utilization took place after the half time of EtOH production (period P_3) then the variation ceased. Contrarily, the acidity remained strain-dependent and connected to variations of alcohol production during the whole process.

The coefficients of polynomial function ($Y=A+[b_1\times X]+[b_2\times X^2]$, where Y is the actual alcohol concentration measured at X hours after initiation of the fermentation) describing the dynamics of ethanol formation during vinification process can be connected to both extracellular and intracellular factors regulating the performance of proper strains, as these coefficients are most probably vector sums of a group of factors. Thus the Constant [A] is a time-independent variable [or group of factors influencing in time-independent manner], and the Primary [b_1] and Secondary [b_2] coefficients [or] and their role in the regulation of alcohol formations can be weighed correlating the strain-dependent coefficients with actual concentration of the components of fermented grape juice as well as the connection between actual concentration of the components and the produced ethanol can be revealed (Table 6). The Constant [A], acting in time-independent manner was not related significantly to strain dependent changes of components of fermented grape juice during the time course of vinification but fructose level (first block of determination coefficients in Table 6). The factors determining the strength of the Primary coefficient [b_1] strongly influenced the utilization of

fructose at the start of ethanol production. In contrast, while these factors took a role in the regulation of actual levels of acetic acid up to the end of fermentation. Altering the formers, the factor group determining the weight of the Secondary coefficient [b_2] strongly influenced the level of all components in the first period (P_1) of fermentation (see Fig. 1). It remained determinative in regulation of the strain-dependent acidity up to the end. We suspect that this group of factors [b_2] was responsible for highly expressed strain-dependent variation in the start of the vinification process.

The identification of these factor groups is a task of the future, and particular experiments should be designed to clarify their nature.

a) Prospects

The wine producers are facing more and more challenges due to the market demands and also the climate change. Recently, there has been an increased demand for wines with a more complex aroma composition spontaneously fermented by natural wild yeast populations. In order to meet this need safely and cost-effectively by wineries, the research of starter cultures has started to focus on the development of *non-Saccharomyces* starter cultures. Today, a number of *non-Saccharomyces* starter cultures are available to allow wineries to model the positive effects of spontaneous fermentation on aroma composition with a safe and controlled method. However, we have little knowledge of how the different species and their mixture affect the process of fermentation, its dynamics. Especially more data requested on their interactions when applied via co- and sequential inoculation. The extreme, unpredictable weather in the last few years has significantly changed the date of harvest compared to the usual times and made it difficult to predict it accurately. These anomalies caused by climate change are new challenges that request appropriate developments in vine cultivation.

In the future, we have to expect the rapid development of synthetic biology. Since cracking the genetic code of the first wine yeast strain (AWRI1631) in 2008, the genomes of several other widely used commercial wine yeast strains – including AWRI1796, EC1118, QA23, VIN7, VIN13, and VL3 – were sequenced and compared with the genomes of laboratory strains of *S. cerevisiae* (S288c and Sigma 1278b) as well as genomes of commercial *Saccharomyces* strains. [49, 70, 71]. The functions of several genes have already been elucidated. With these results, we can equip the yeasts with advantageous and valuable properties for industrial use. For example, the *FSY1* and *MPR1/2* genes are thought to convey fermentation robustness and performance; the *IRC7* gene might be associated with aroma enhancement in wine. *IRC7*-expressing strains seem to release more volatile thiols during fermentation, thereby increasing the fruitiness of the wine. [49, 72].

We would facilitate the planned scheduling of the grape processing and winemaking with our ongoing work. If we know the analytical parameters of our raw material and we know the fermentation ability of the starter culture we want to apply well, we can predict the duration of fermentation as accurately as possible. After further experiments, it is necessary to develop new models to be able to predict the dynamics of fermentation more and more accurately, calculated with the effect of more sophisticated winemaking methods. The different inoculation methods, the interaction of different yeast species and their mixtures, the supply of nutrients, and the regulation of fermentation cycles at different temperatures affect the whole fermentation, including the time of its duration.

IV. Conclusions

The strain-dependent variations of the dynamics of ethanol production during the vinification process can be reliably characterized with second-order polynomial function (Table 3) that has significant predictive power ($p<0.05$) for calculation of parameters such as Lag-phase, Half time, Endpoint and Specific Intensity of ethanol production. A further advantage of this function is the possibility to weigh the role of constant, primary and secondary effects as well as to analyze their relationships in a strain-dependent manner (Table 4).

Although some quantitative differences manifested between *Saccharomyces* and *Starmerella bacillaris* strains, more *non-Saccharomyces* strains should be involved in studies to make satisfying conclusions in this respect.

Most probably, the toxic effect of ethanol produced also affects the strain-dependent dynamics of fermentation, primarily in the last phase nearing the End point (P_4 on Fig. 1), and this sensitivity response may influence the interaction of factors regulating both the ethanol production and the composition of the new wine; however, this assumption needs further studies. Nevertheless, the strain-dependent counteractions of constant [A] and time-dependent factors ([b_1] and [b_2]) play a seemingly more intensive role in the regulation of ethanol production in the first half of the vinification process (P_1 and P_2 stages, see Fig 1). In this period, the alcohol concentration is lower than 6 percent, and we can assume that the possible autocidal effect is not playing a role yet contrary to later phases (P_3 and P_4 stages, see Fig 1).

Near linear trend was manifested as well as the position of strains fits well independently on their age and taxonomic position when interactions of regulating factor groups ([A], [b_1] and [b_2]) were compared (Fig 9). Further studies are requested for an explanation of this finding.

Today we are not living in a time when we can be satisfied with routinely applied technologies if we want to run our winery successfully and economically on a market with a constant oversupply. We need to equip ourselves with the latest and most in-depth knowledge to gain an advantage. With the development of gene technologies, the range of possibilities can only be limited by our imagination.

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Table 1: Potential of non-*Saccharomyces* yeast strains to improve wine quality

Sequential yeast used	Reference	Sequential yeast used	Reference
Candida cantarellii	[73]	*Lachancea lanzarotensis*[d]	[74]
Candida stellata	[75]	*Lachancea thermotolerans*	[74]
Candida zeylanoides	[76]	*Metschnikowia pulcherrima*	[77, 76]
Candida zemplinina[a]	[17]	*Pichia fermentans*	[78]
Debaryomyces vanriji[b,g]	[79, 80]	*Pichia guilliermondii*	[81]
Hanseniaspora guilliermondii	[82]	*Pichia membranifaciens*	[81]
Hanseniaspora osmophila	[83]	*Schizosaccharomyces pombe*	[52, 53]
Hanseniaspora uvarum	[82]	*Torulaspora delbrueckii*[a]	[17, 76]
Hanseniaspora vineae[c]	[84]	*Wickerhamomyces anomalus*[f]	[85]
Kloeckera apiculata	[86, 87]	*Williopsis pratensis*	[76]
Kluyveromyces marxianus	[81]	*Williopsis saturnus*	[88, 89, 90]
Lachancea fermentati[d]	[91]	*Zygosaccharomyces bailii*	[76]

Strains of listed species have been involved in experiments in the past two decades and their effects on wine quality have been elucidated in cited publications.

[a]Appropriate for carrying out the alcoholic fermentation [17]; [b] β-glucosidase activity; [c]concurrent; [d]H_2S production; [e]malic acid conversion to ethanol; [f]cider fermentation; [g]syn: Candida famata.

Table 2: Kinetic parameters of the fermentation dynamics of wine yeast strains

Strains[a]		Oenological parameters [b]						
Code	Source	Type[f]	L.P.	H.T.	H-L[g]	SEP[h]	DC[i]	EtOH[j]
Saccharomyces cerevisiae (Desm.) Meyen								
10-157	type strain of *S. c.*	B	142	301	160	0.517	0.998	11.65[a]
10-1390	com. starter culture	E	115	200	85	0.911	0.995	12.25[c]
10-1343	Young wine	A	151	276	125	0.797	0.974	12.60[gh]
10-1346	Young wine	A	100	187	87	0.965	0.997	12.85[kl]
10-1352	Young wine	D	74	191	116	0.686	0.989	12.25[c]
10-1345	Young wine	A	115	234	119	1.396	0.987	12.45[de]
10-1347	Young wine	A	105	204	98	1.022	0.971	12.85[kl]
10-1350	Young wine	C	96	191	95	0.941	0.978	12.75[jk]
10-1357	Muscat Lunel wine	D	85	169	85	0.799	0.995	12.55[fg]
10-1358	Young Furmint wine	B	114	261	147	0.662	0.989	12.20[bc]
10-1348	Wine sediment	A	174	282	108	0.777	0.950	12.15[b]
10-1349	Wine sediment	A	151	258	107	0.971	0.971	12.70[ij]
10-1355	Wine sediment	A	120	259	139	0.897	0.990	12.90[lm]
10-1351	Wine sediment	A	110	226	116	0.902	0.987	12.65[hi]
10-1354	Furmint sediment	C	104	182	78	1.059	0.997	12.85[kl]
10-1344	Furmint sediment	D	95	166	71	0.975	0.996	12.45[de]
10-1353	Furmint sediment	A	101	198	97	0.939	0.995	12.45[de]
10-1356	5-year old aszú wine	B	98	199	101	0.875	0.994	12.95[m]
10-1359	5-year old aszú wine	C	102	187	85	0.805	0.968	12.60[gh]
10-489	sweet botrytized must[c]	n.d.	79	108	29	2.001	0.993	12.69[ij]
10-493.	sweet botrytized must[c]	n.d.	57	107	49	1.305	0.999	12.89[lm]
Saccharomyces uvarum Beij								
10-486	sweet botrytized must[c]	n.d.	49	78	29	1.610	0.974	12.52[ef]
10-499	sweet botrytized must[c]	n.d.	65	98	32	1.898	0.992	12.66[hi]
Starmerella bacillaris (Kroemer & Krumbholz) F.L. Duarte & Á. Fonseca								
10-374	sweet botrytized must[d]	n.d.	104	148	44	1.649	98	12.68[i]
10-5-11	Botrytized grape[e]	n.d.	99	104	6	1.708	89	12.44[d]

[a]All strains but 10-157 [ATCC 26108] and 10-1390 [Uvaferm 43; Lallemand Inc., Montreal, Canada] were isolated in Tokaj Wine region and deposited in the collection of Department of Genetics and Applied Microbiology of University of Debrecen. Data on the origin and oenological properties of 10-1343 to 10-1358 were delineated by Kállai et al. [61]. [b]Abbreviations: L.P. and H.T. = lag phase and half time (hours). [c]Strains isolated of botrytized grape must by Antunovics et al. [30]. [d]Strain was isolated identified by Sipiczki [92]

[e]Strain was isolated and identified by Csoma and Sipiczki [93]. [f]Type of strains according similarities in oneological properties (see Fig. 7). [g]Time (hours) requested to produce half of the final ethanol concentration since the end of lag phase. [h]Specific ethanol production (SEP) produced at half time (mM h^{-1}). [i]Determination coefficients of regression curves used for calculation of parameters (see Fig. 1). [j]Ethanol concentrations in new wines, the percentage values (v/v) labelled by the same letter are not different at $p<0.05$ level ($LSD_{0.05}=0.075$, $F=18.2$).

Table 3: Similarity of kinetic parameters calculated by various functions

Matrix A	Matrix B								Limits (h)	
	MAN	SIG	GOM	PRO	SAT	LOG	HYP	POL	min	max
Manual	0.38	0.380	0.414	0.327	0.596	0.462	0.494	0.768	57	174
Sigmoid	0.287	0.63	0.565	0.676	0.250	0.838	0.824	0.859	0	126
Gompertz	0.005	0.026	0.01	0.527	0.083	0.668	0.719	0.762	2	97
Probit	0.003	0.012	0.210	0.01	0.242	0.803	0.795	0.856	15	70
Saturation	0.633	0.208	0.002	0.023	0.47	0.323	0.370	0.620	88	287
Logarithmic	0.717	0.390	0.017	0.057	0.602	0.95	0.970	0.954	16	120
Hyperbolic	0.693	0.330	0.004	0.085	0.559	0.936	0.98	0.968	23	126
Polynomial	0.925	0.290	0.001	0.328	0.315	0.756	0.904	0.70	55	121
Limits (hours)										
minimum	102	71	130	129	107	66	59	67		
maximum	296	394	301	297	330	296	275	294		

Determination coefficients of regression between values of lag phases (time requested for start of detectable production of ethanol, etap P_1 on Fig 1) and half times (etap P_2 on Fig 1) of fermenting S. cerevisiae strains (N=21) calculated by log/probit, Baule Mitcherlich (saturation), logarithmic, hyperbolic and polynomial functions or obtained by manual fitting. The variations in fitness of named approaches were demonstrated in Figure 3.

Matrix A – lag phases (the limits are shown in vertical columns), Matric B – half times (the limits are in last lines), while determination coefficients related to the similarities between lag phases and half times calculated by the same function (two digits) are in diagonal cells (r^2=0.179, $p<0.05$; r^2=0.288, $p<0.01$; r^2=0.426, $p<0.001$).

Table 4: Connection between etaps of fermentation[a] and strain dependent factors of polynomial functions describing dynamics of the ethanol production

Variable (D)	Importance of factor groups[b]			Parameters of the equation[c]			
	β_C	β_P	β_S	Chi-sqr.	R-sqr.	p	λ Prime
EtOH conc.[d]	0.2217	-0.2762	0.3048	11.75	0.4210	0.0083	0.5790
Lag phase[e]	0.0044	0.1517	-0.2633	26.54	0.7090	7.4E-06	0.2910
Half time[f]	-0.2875	0.4331	-0.5323	62.51	0.9454	1.8E-13	0.0546
H-L[g]	-0.5188	0.6451	-0.7267	58.18	0.9332	1.5E-12	0.0668
ISEP[h]	0.4147	-0.5527	0.6448	28.26	0.7314	3.2E-06	0.2686

[a]=See Fig. 1. [b]= Coefficients (β_C, β_P, β_S) of the functions ($D_{1\text{-}25}=\{[CSQ]_{1\text{-}25}+[PSQ]_{1\text{-}25}+[SSQ]_{1\text{-}25}\}$), where $D_{1\text{-}25}$= dependent variable; $CSQ_{1\text{-}25}$=Constant; $PSQ_{1\text{-}25}$=Primary; $SSQ_{1\text{-}25}$=secondary coefficients of the polynomial functions of 25 strains describing the dynamics of their ethanol production, respectively;

[c]= Parameters of the multiple linear regression function: $D=f(X_1,X_2,X_3)$, where D is a dependent variable of the first column.

[d]=Ethanol concentrations in new wines fermented by proper strains listed in Table 1. Data imported of Kállai et al. [61]; [e]= Strain dependent Lag phases (hours) of EtOH production. [f]= Strain dependent time (hours) requested to reach the 50% of the final EtOH concentration produced by proper strains as measured of the start of fermentation.

[g]= Time (hours) requested to reach the 50% of EtOH by proper strains of the start production. [h]= Specific rate of alcohol production (mol EtOH/hour);

Table 5: Time dependent influence of strain characters on dynamic changes in composition of the fermented grape juice

Parameters	Time course (days)						F_{repl}
	5	10	15	20	25	30	
Glucose	0.9216***	0.5680+	0.0704-	0.0005-	0.0002-	0.0189-	0.53
Fructose	0.9742***	0.9047***	0.6837+	0.1304-	0.0031-	0.0084-	0.04
TS-TF-TG	0.8520**	0.0535-	0.0046-	0.0606-	0.4820-	0.3654-	
Acetic acid	0.8179**	0.7715*	0.9105***	0.8591*	0.8545**	0.8866**	1.88
TA-AA	0.9920***	0.9913***	0.9823***	0.9808***	0.9769***	0.9894***	
pH	0.9961***	0.9974***	0.9959***	0.9948***	0.9985***	0.9972***	0.03

The concentrations of components measured (parameter) by the given time course were imported from Kállai et al. [61], and used for calculations applying multiple regression analysis to reveal the connection between dynamics of ethanol production and changes in composition of the fermented grape juice. TS-TF-TG=[total sugar]-[glucose]-[fructose], TA-AA=[total acids]-[acetic acid].

*The R^2 is the determination coefficient of the function $P_{(1-21)}=[C_{(1-21)}+b1_{(1-21)}+b2_{(1-21)}]$ where $P_{(1-21)}$ is the parameter measured at the time of sampling and the $[C_{(1-21)}+b1_{(1-21)}+b2_{(1-21)}]$ are coefficients of proper functions describing the dynamics of alcohol production of each strain [n=21] $EtOH_{(0-30)}=C+b_1X+b_2X^2$ describing the dynamics of ethanol production (Figure 3). We call the cases strain dependent where the coefficients were labelled with symbols + (p=0.05-0.1), *(p=0.01-0.05), **(p=0.001-0.01), ***(p<0.001) and strain independent with - (p>0.1). The F values show the exactitud of the measurement of the parameter concerned $F_{0.1}=3.18$).*

The values of DCs are proportional to dependence of strain properties related to dynamics of ethanol production, and values lower than 0.5 might be considered as low importance of proper strain characters in this respect. For example, changes in glucose level were strain dependent only in first etaps of vinification (P_1 and P_2 in Fig. 1), and the number of stars marks the strength of effect.

Table 6: The influence of factors regulating strain dependent dynamics of ethanol production on the actual level of components in fermented grape juice.

Factors	Components	Time course (days)					
		5	10	15	20	25	30
Constant (A)	Glucose	0.181	0.018	0.014	>0.001	>0.001	>0.001
	Fructose	0.682	0.172	0.016	0.003	0.001	0.003
	TS-TF-TG	0.029	0.038	0.019	0.005	0.050	0.076
	Acetic acid	0.459	0.332	0.395	0.312	0.274	0.447
Primary (b1)	Glucose	0.416	0.001	0.005	>.0.001	>0.001	>0.001
	Fructose	0.813	0.427	0.094	0.017	0.002	0.004
	TS-TF-TG	0.175	0.065	0.027	0.000	0.131	0.123
	Acetic acid	0.548	0.402	0.545	0.444	0.438	0.575
Secondary (b2)	Glucose	0.610	0.049	>0.001	0.001	0.002	0.001
	Fructose	0.880	0.639	0.237	0.049	0.002	0.004
	TS-TF-TG	0.388	0.101	0.034	0.014	0.254	0.171
	Acetic acid	0.603	0.428	0.645	0.541	0.575	0.661

Determination coefficient (DC) of multiple regression (time changes in the level of component given versus proper coefficient) higher than 0.5 mark selective and significant effect (p<0.05) of the strain dependent factor group (underlined). The values of DCs are proportional to dependence of strain properties related to dynamics of ethanol production, and values lower than 0.5 might be considered as low importance of proper strain characters in this respect. For example, changes in glucose level were strain dependent only in first etaps of vinification (P_1 an P_2 in Fig. 1).

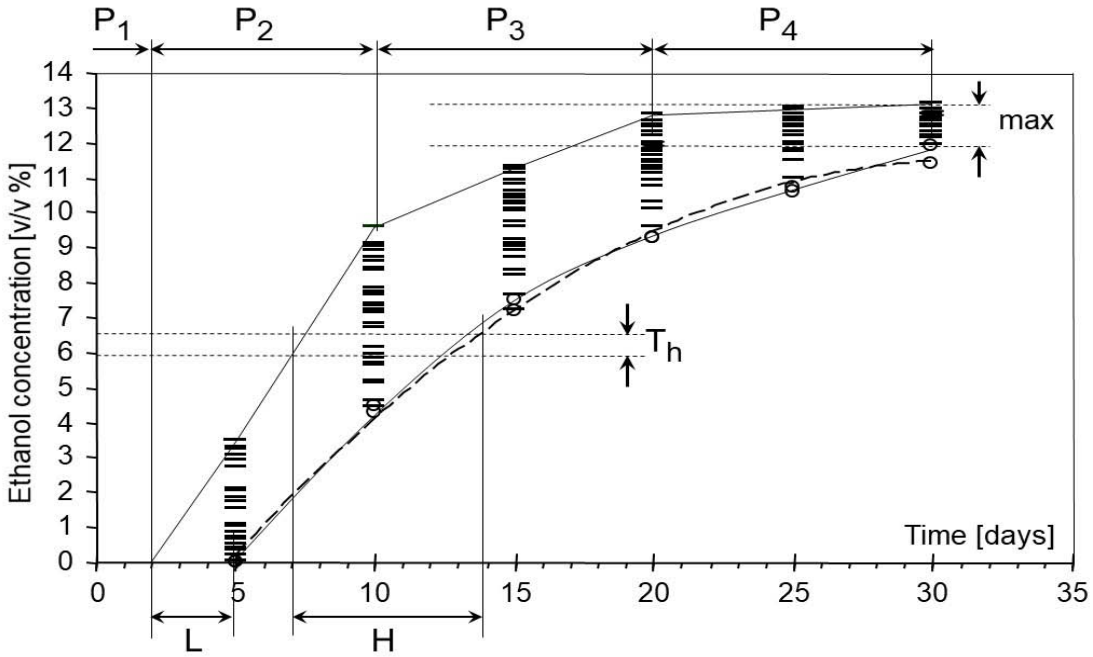

Figure 1: Time dependent changes of ethanol concentration during the fermentation of grape juice

The amounts of ethanol produced by reference strain [ATTC 26108] were marked with opened circles, while those of *S. cerevisiae* strains described by Kállai et al. [61] were marked with short lines. Full lines drawn manually show kinetics of changes in ethanol production (AC) of less and most potent strains (48 and 52), respectively, while the stripped curve was fitted to plotted experimental data of reference strain with function $AC(\%) = -0.0167[Time]^2 + 1.0361[Time] - 4.57$ ($R^2 = 0.9964$).

The process of alcoholic fermentation can be divided into four periods; P_1 – no measurable amount in the medium (lag phase), P_2 – accelerating growth of concentration, P_3 – near monotonous growth, P_4 – retarding growth. The arrow **L** marks the interval between lag phases of less to most rapid strains, while the arrow **H** marks the interval of the time requested to rich the half of produced ethanol concentration of less to most potent producers in the set of strains examined, and correspondingly; *max* is the range between lowest and highest ethanol concentrations in new wines, meanwhile T_h is the half of former values.

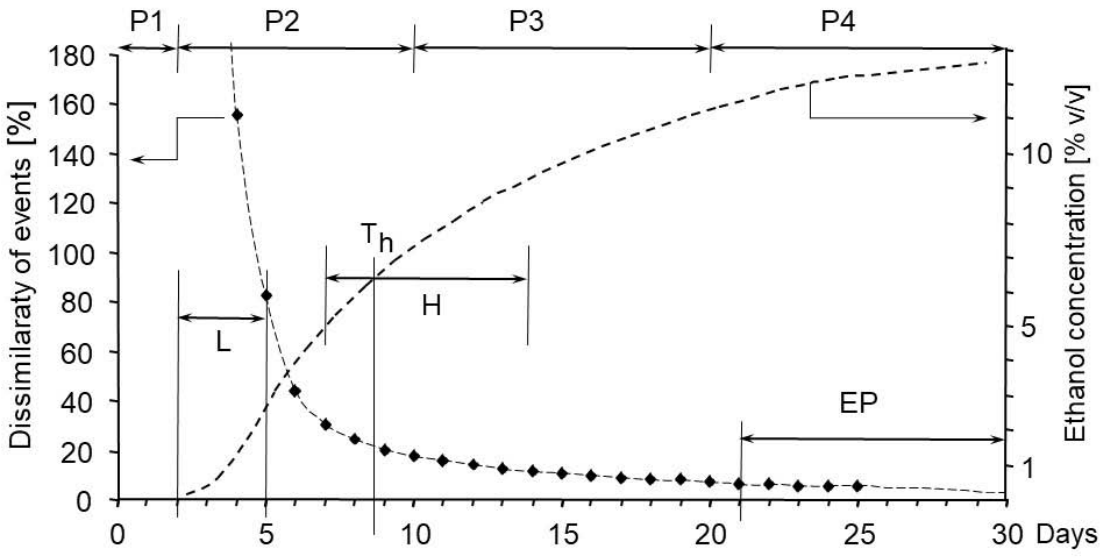

Figure 2: Changes of varietal differences in production of ethanol during fermentation

The broken line markes time dependent average values of actual ethanol concentration in the medium (coordinate at right side). The time dependent variation of ethanol production (diamonds, coordinate at left side) relates to dissimilarity of fermentation capacity of *S. cerevisiae* strains (n=19) at the given sampling time.
Abbreviation: P_1, P_2, P_3 and P_4 at the top of graph are intervales distinguished in Figure 1.

Arrows L, H and EP imported from Figure 1 show the strain dependent variation (minimum to maximum) of lag phase, half time and end point. T_h = The average half time of the set of *S. cerevisiae* strains isolated in Tokaj region.

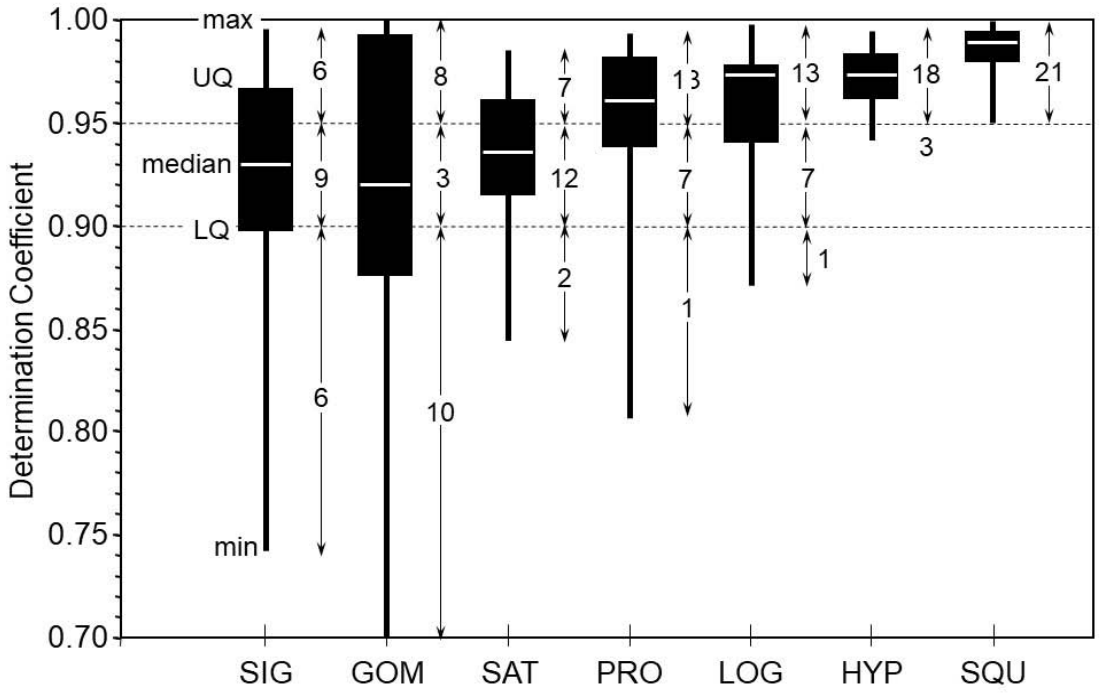

Figure 3: Box & whiskers plot of determination coefficients of functions applied for extrapolation of kinetic parameters of the alcohol fermentation by *S. cerevisiae* strains

Box and whiskers plots were constructed of the determination coefficient values of the curve fittings based on experimentally measured ethanol concentration in samples taken during the fermentation in the medium of 21 *S. cerevisiae* strains, applying logistic (SIG), Gompertz (GOM), Baule-Mitcherlich (SAT), probit (PRO), logarithmic (LOG), hyperbolic (HYP) and polynomial (SQU) functions.

The higher coefficients show the higher significance of similarity between calculated and experimentally determined ethanol concentrations in the medium. Numbers at the right side of boxes in vertical arrows show cases that could be fitted at $p>0.1$, $0.1>p>0.05$ and $p<0.05$ probability levels.

Abbreviations: maximum (max) and minimum (min) values, lower (LQ) and upper (UQ) quartiles, the white line is the median in the black box that shows the size of the middle two quartiles.

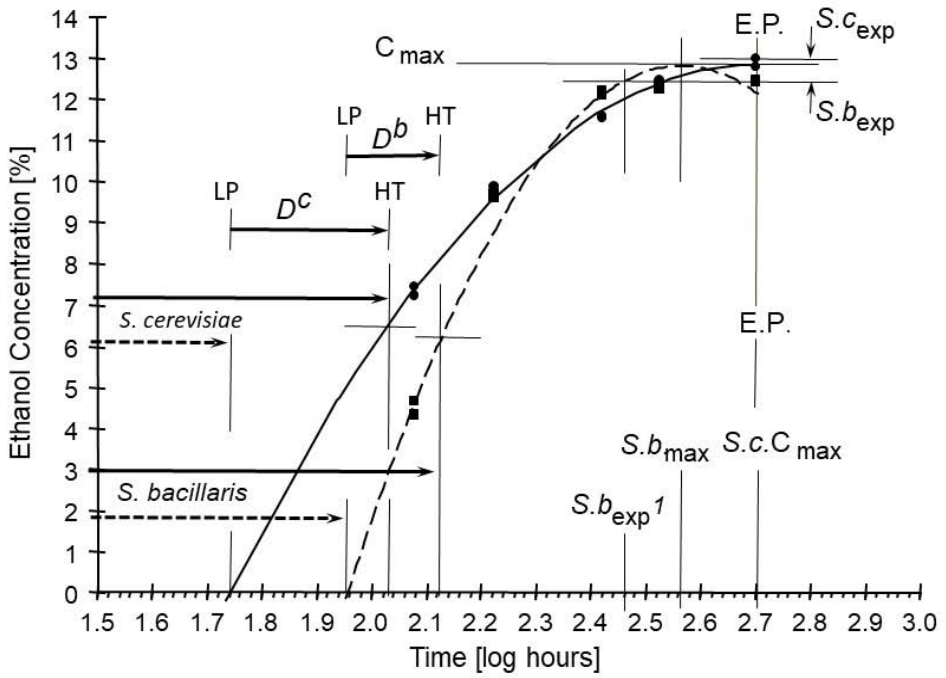

Figure 4: Dynamics of ethanol accumulation during vinification

The curves were calculated of experimentally determined ethanol concentrations (AC) in the medium (measured values at subsequent samplings of *S. cerevisiae* [10-486] and *St. bacillaris* [10-374] are marked with full circles and squares, respectively):

S. cerevisiae; AC(%)= -0,0167[Time]^2 + 1,0361[Time] - 4,57 (R^2 = 0,9964)

St. bacillaris; AC(%)= -0,0167[Time]^2 + 1,0361[Time] - 4,57 (R^2 = 0,9964)

Abbreviations: LP=Lag phase, HT=Half time, E.P.= End point, D= time requested to produce half of the final ethanol content by strains, C_{max}=the maximum ethanol concentration calculated, $S.c._{exp}$ and $S.b._{exp}$=ethanol concentration measured at the end (E.P.) of fermentation, $S.c._{max}$ and $S.b._{max}$= ethanol concentration measured at the end (E.P.) of fermentation, $S.b._{exp}{}^{1}$= time requested to produce ethanol concentration measured at the end point (this value for *S. cerevisiae* equal with calculated one).

The bold arrows indicate points of the end of lag phase (LP) and the half time (HT) of *S. cerevisiae* (full line) and *St. bacillaris* (stretched line); the values were compiled in Table 1.

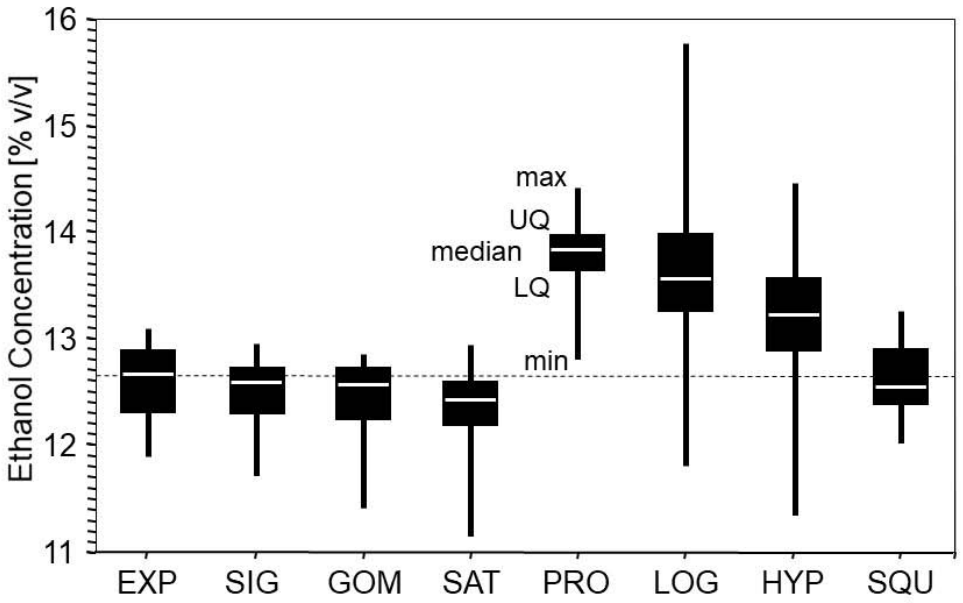

Figure 5: Box & whiskers plot of either measured or extrapolated ethanol concentrations of new wines fermented by *S. cerevisiae* strains of Yellow Muscat must

The concentration of ethanol measured in new wine (EXP) was taken as a standard for comparison of concentration values extrapolated applying logistic (SIG), Gompertz (GOM), Baule-Mitscherlich (SAT), probit (PRO), logarithmic (LOG), hyperbolic (HYP) and polynomial (SQU) funcions. Box and whiskers plots were constructed of the measured values (EXP) or extrapolated ethanol concentration at the end of fermentation in the medium of 21 *S. cerevisiae* strains. The dotted line shows the median of analytically measured values.

Abbreviations: maximum (max) and minimum (min) values, lower (LQ) and upper (UQ) quartiles, the white line is the median in the black box that shows the size of the middle two quartiles.

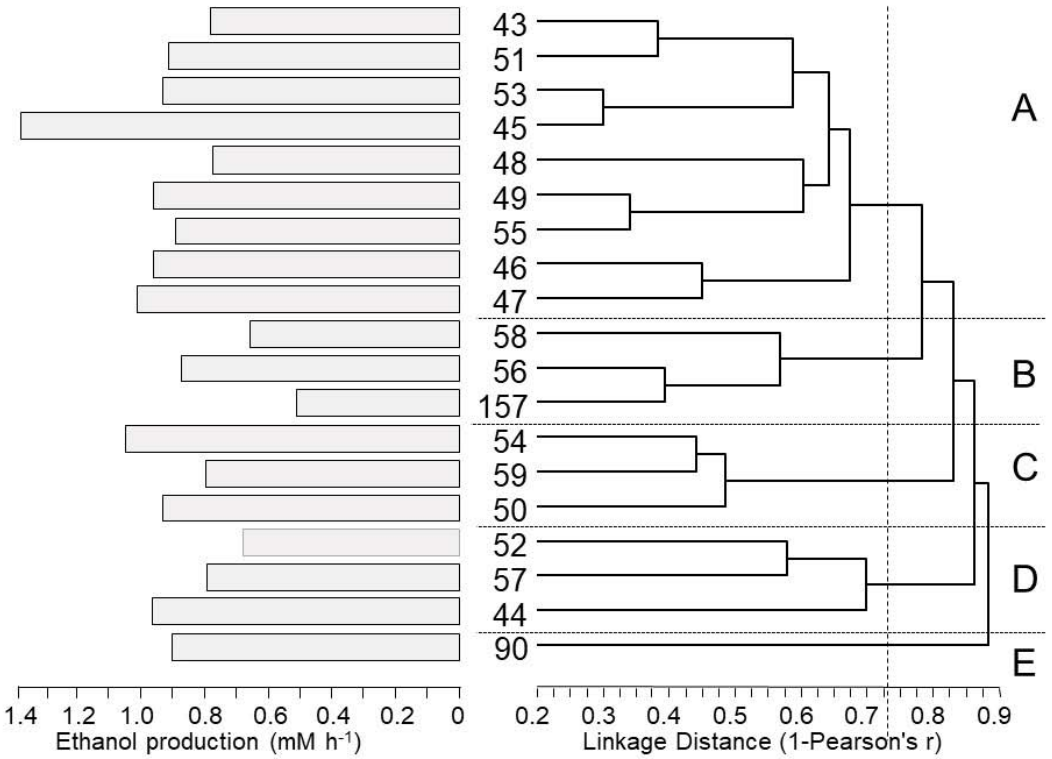

Figure 6: Relationships between specific rate of ethanol production and similarities of oenological properties of *S. cerevisiae* strains

The number of strains correspond to that in Table 2. The specific rate of ethanol production (mM h^{-1}) was calculated in half time applying polynomial function (see Table 2).

The clustergram was computed of data published by Kállai et al. [61]. The strains comprised in subcluesters differ in their oenological properties at $p<0.05$.

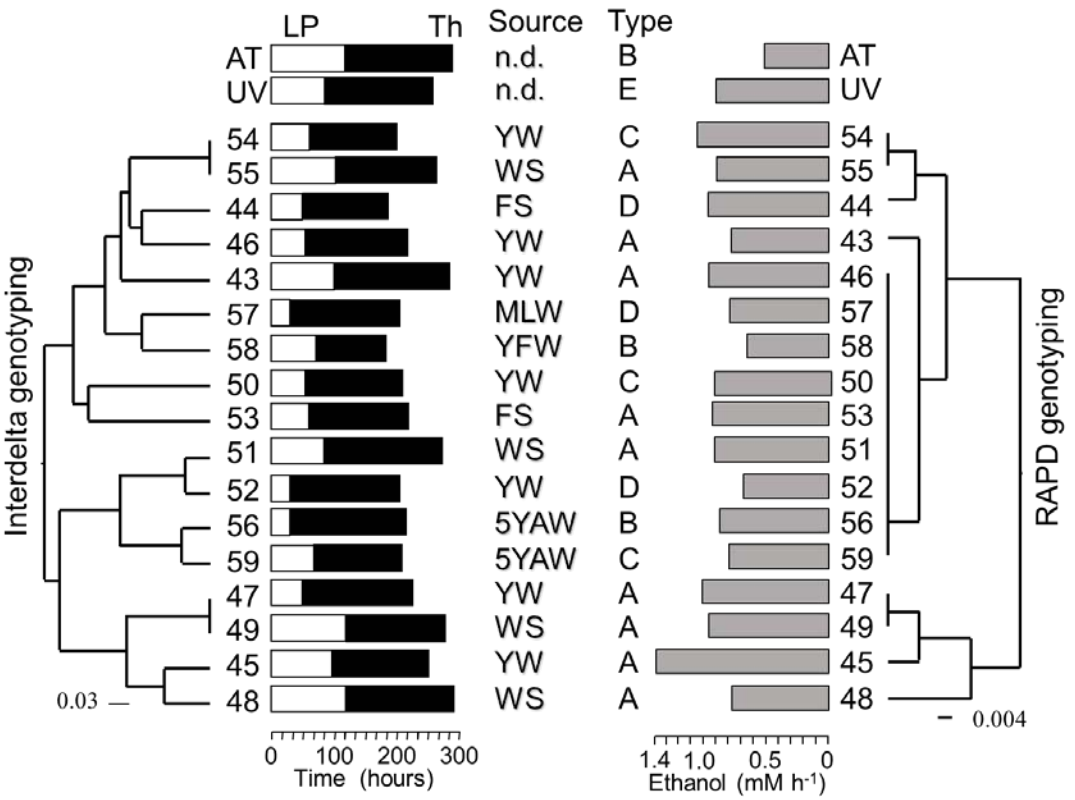

Figure 7: Relationships between specific ethanol production rates of strains and their genetic variability established on the base of molecular diagnostics

The clusterograms have been imported of our earlier work *Kállai et al.* [61], either interdelta (left side) or RAPD (right side) method was applied for elucidating the molecular diversity of strains.The number of strains corresponds to the last two numerals in codes of strains given in Table 1. Columns assigned to the left graph indicate time intervals of the inoculation to the evolution of detectable ethanol concentration (Lag phase, white prism) and subsequent period to produce 50 % of the final alcohol content in new wine fermented by the given strain (black prism), while the others at the right side are proportional to specific rate of ethanol production calculated at T_h of the strain concerned, with polynomial function ($p<0.05$).

Abbreviations: AT=type stain [ATCC 26105] and UV=commercial starter strain [UVA43]; L = Lag phase, T_h = half time; Source: YW = young wine, WS = wine sediment, FS = Furmint sediment, MLW = Muscat Lunel wine, YFW = young Furmint wine, 5YAW = 5 years old aszu wine; Types: A → D = Subclusters (See Fig. 7); Bars: the genetic distances according to *Kállai et al.* [61].

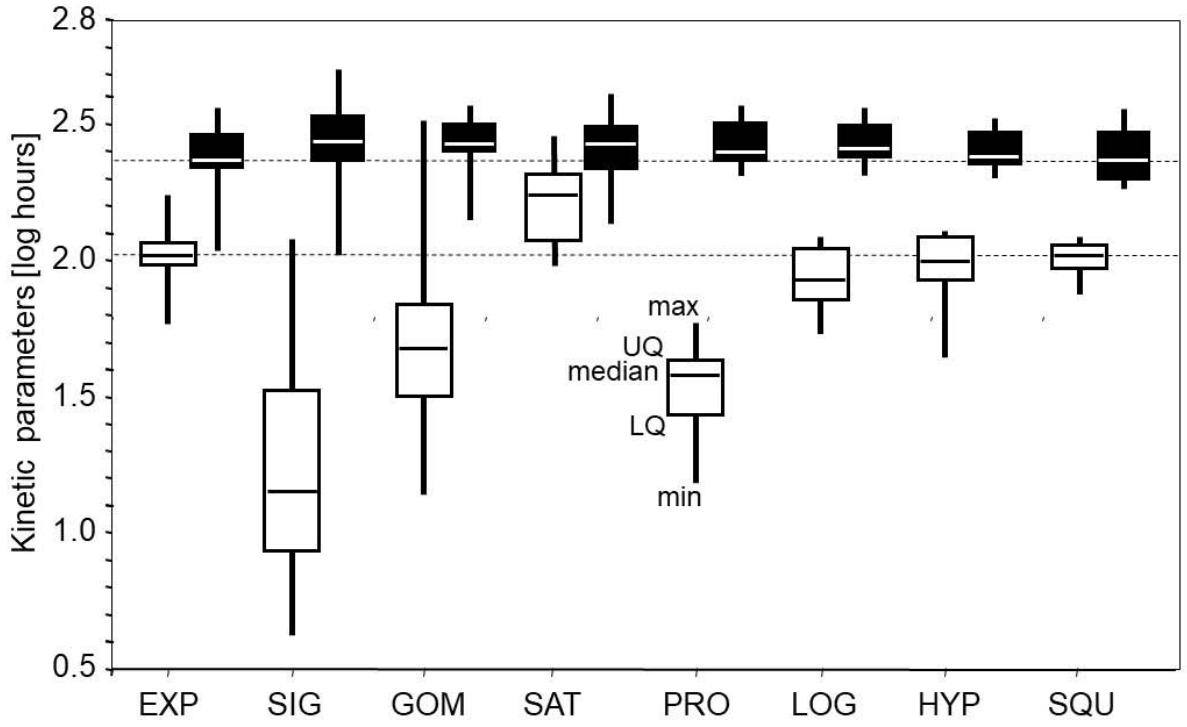

Figure 8: Turn points in alcohol production

The lag phase (white box) and half time (black box) of ethanol production extrapolated either manually (EXP) or applying logistic (SIG), Gompertz (GOM), saturation (SAT), probit (PRO), logarithmic (LOG). hyperbolic (HYP) and polynomial (SQU) funcions. Abbreviations: maximum (max) and minimum (min) values, lower (LQ) and upper (UQ) quartiles, black or white lines are the median in white or black boxes, respectively. The boxes show the size of the middle two quartiles.
The horizontal dotted lines indicate either the median of manually fitted values of lag phases or half times.

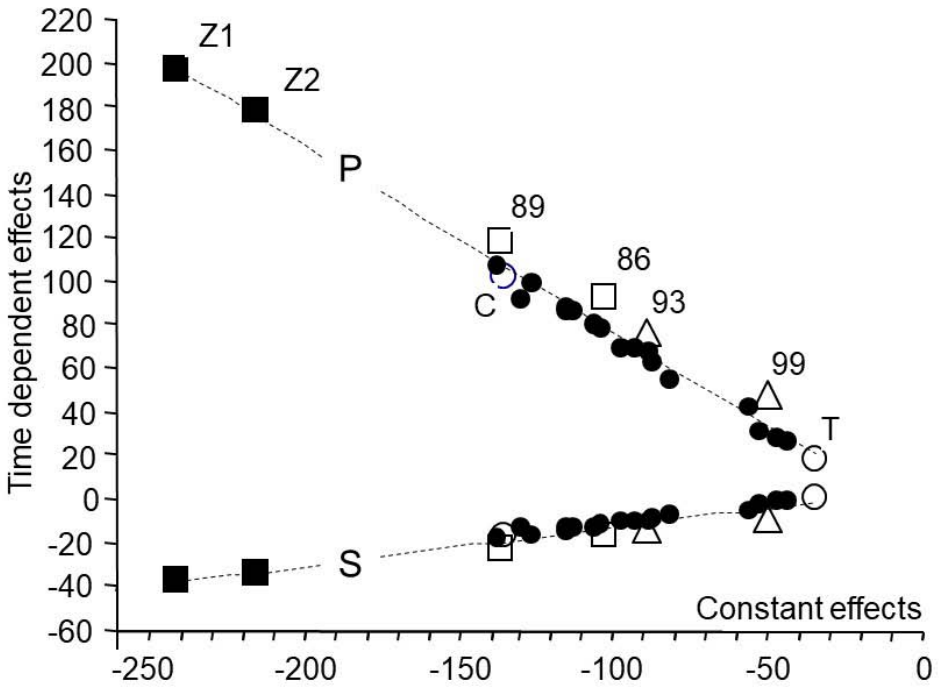

Figure 9: Relationships between constant and time dependent factors influencing the strain specific ethanol production

The regression lines P (Pi = -0.8585Ci - 9.7445, r^2=0.98) and S (Si = 0.1795Ci + 5.0738, r^2=0.94) mark relationship between time dependent primary and secondary factors *versus* time independent constant factors influencing intensity of actual ethanol production of strains (i).

The labels correspond to the last two numerals of strains in codes given in Table 2. Abbreviations: T=type stain [ATCC 26105] and C=commercial starter strain (opened circles) of *S. cerevisiae* (closed circles) fermented Yellow Muscat must in laboratory models, while *S. cerevisiae* and *S. uvarum* strains (opened squares and triangles, respectively), and Z1[93] and Z2 [30] strains of *St. bacillaris* (full squares) fermented Furmint grape must in a winery.

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Adaptability and Stability of Elite Potato (*Solanum Tuberosum.* L) Genotypes in Kenya

By Angwenyi Maobe, Kahiu Ngugi, Thiago Mendes & Richard Nyankanga
University of Nairobi

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Keywords: adaptability, GGE biplot; stability and tuber yield.

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I. Introduction

Potato (*Solanum tuberosum* L.) is an important food security crop and a source of income worldwide (Muthoni and Hussein 2018). In Kenya, the crop is grown by approximately 800,000 small scale farmers on more than 158,000 ha of land per season with a yield estimate of 1.2 million tons (Riungu, 2011). This harvest is worth about KES 13 billion at farm gate level and KES 40 billion when it is at the consumer level (Muthoni and Hussein 2018). The Kenyan farmers produce both Processing and Table types in the Central highlands of Kenya, Bomet, Molo, Narok and Meru where most of them have occupied approximately 25% of their land with potato production. Recently, there has been a decline in potato production in Kenya mainly due to lack of adaptive and stable cultivars, lack of clean seeds, poor pest and disease management practices and less competitive marketing strategies (Riungu., 2011; and Muthoni et al., 2015).

International Potato Center (CIP) has for a long time led the potato improvement strategies in Kenya through screening and evaluation of imported cultivars especially from Europe. To achieve higher and more stable tuber yields, selection in the target environment is necessary (Muthoni and Hussein 2018). The challenges of increased potato production in the country are compounded by decreased land hectarage and inadaptability of introduced cultivars to local growing environments (Gildemacher et al.,2009; Bai, et al.,2014). Importation of cultivars, has led to inconsistent genotypic expression in the diverse environments which in turn prolongs the selection process because of genotype by environment interactions (G x E) (Muthoni et al.,2015).

There is need therefore to ascertain the levels of G x E interactions exhibited by elite potato genotypes being developed and assess their adaptability, stability and yielding potential. In order to do this, dependable analytical methods that would identify the magnitude of G x E interactions are needed to determine the levels of genotypic main effects and environmental influence. External factors from the environments need to be estimated and measured to determine their individual contribution (Gauch and Zobel 1996).

Parametric methods have been used before to measure the effects of G x E interactions, but have proven to be less informative since they are based only on analysis of variance (ANOVA). Multivariate analytical methods such as Additive Main Multiplicative Interaction (AMMI) and Genotype, Genotype Environments (GGE) that analyze both the genotypic effect and explain the interaction using ANOVA and Principal Component Analysis (PCA) respectively, provide more robust information on the status of G x E interactions.

Using graphical bi-plots, these methods provide information that could be relied on to draw major conclusions and recommendations about the environment and the genotype (Ani et al., 2016). The bi-plots are based on the first and second principal components (PCA1 and PCA 2) that are derived from singular value decomposition (SVD) of the environment centered data. The GGE bi-plots identify such aspects as, suitability of locations for genotypes in the 'which won where' and determine the discriminating ability and representativeness of locations (Yan et al.., 2007). The bi-plots also provide information on mega environments which play a key role when pooling of information on similar environments is necessary to reduce the cost of evaluation (Affleck et al., 2008). The GGE methodology is also capable of providing details on the qualitative aspects of the yield in relation with the environment (Bach et al., 2012).

Development of superior genotypes is disadvantaged by the lack of clear description of the

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environments and adaptability aspects of the genotypes. Mega environments are important in identifying similarities and differences of test environment, provides information about the adapted genotype and ultimately establishing the yield potential of a genotype in a given region (Yan and Rajcan., 2002; Kalidasu et al., 2016). The mega environments provide important information that enables prudent resource utilization without compromising the quality of the information obtained. The potato variety breeding efforts in Kenya have developed elite genotypes whose stability and adaptability has not been established before.

II. Materials and Methods

The experiments were conducted in six locations namely, Molo, Narok, Cherengangy, Burnt Forest in Rift Valley, Timau and Kibirichia during the national performance trials in 2015 and 2016 long rainy seasons (Table 1 and Fig.1). The sites were selected from zones known for potato production in Kenya and they represent mid to high altitude agro-ecologies. These sites receive varying amounts of rainfall as well as temperatures and experience a bimodal rainfall pattern annually as shown in Table 1.

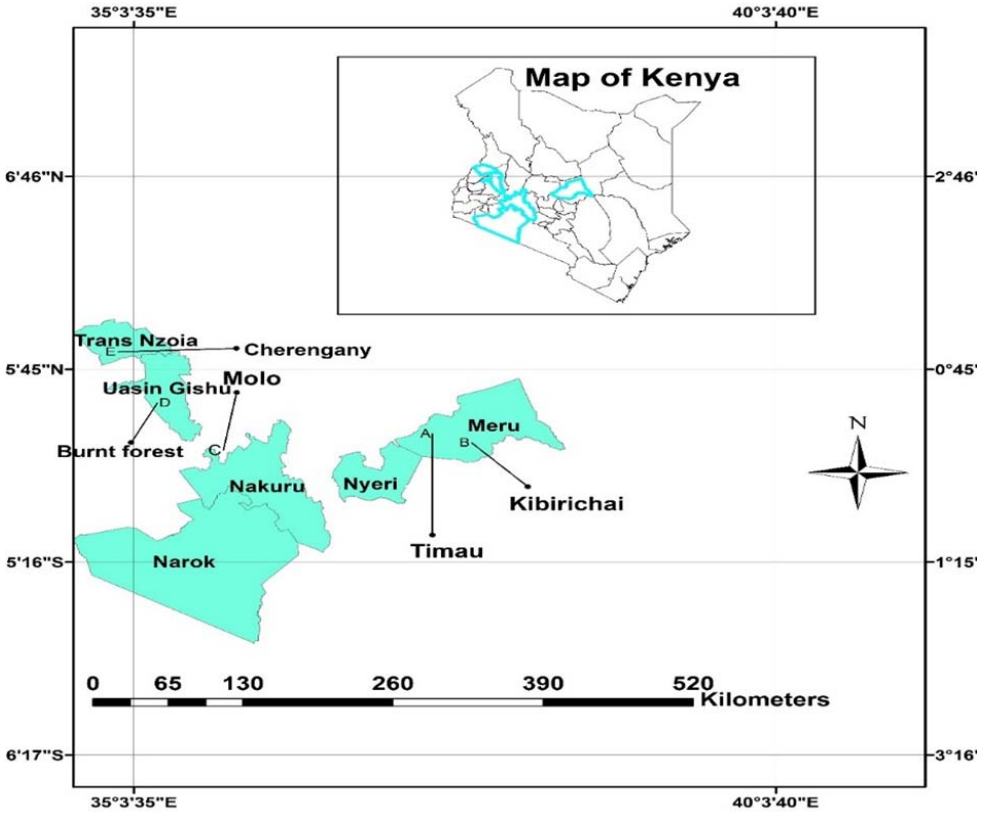

Figure 1: Map of Kenya showing the evaluation environments

Table 1: Geographical and climatic description of selected trial sites in Kenya

Location	Altitude (masl*)	Annual rainfall	Temperatures	
			Minimum	Maximum
Molo	2506	1131	16	24
Narok	1827	771	9.2	26
Cherangany	2,047	1,200	14	30
Burnt Forest	2419	1103	12	25
Timau	1767	587	6.9	23.3
Kibirichia	1827	24	16	24

**masl = Meter above sea level. Source: Kenya National Meteorological Agency, 2015*

Three Table type commercial varieties namely, G20 (Shangi), G11 (Kenya Karibu), G24 (Tigoni) and one Processing type, G8 (Dutch Robjyin) were used as checks. Among the 23 genotypes, ten were Processing types (G1, G2, G3, G10, G12, G13, G14, G17, G22 and G26) whereas thirteen were Table types (G4, G5, G6, G7, G9, G15, G16, G18, G19, G21, G23, G25, and G27). In each location, the seed was planted and managed using the farmer potato production practices. The trials were laid down in a randomized complete block design (RCBD)with plots measuring, 3 meters by 3 meters, with 0.30 meters between the plants, 0.75 meters between rows and 1meter between plots. Fifty sprouted seed potato tubers were planted per plot making a plant population of 1,350 plants per block. The seed tubers were planted at a depth of 10cm with application of Di-ammonium Phosphate fertilizer (DAP) at a rate of 500 Kg per hectare (Muthoni et al., 2016). The stems were cut off at 90 days after planting and harvested 15 days later after tuber hardening. Tuber yield was scored first in kilograms per plot but converted to tons per hectare.

III. Data Analysis

Tuber yields for each genotype and location were subjected to analysis of variance (ANOVA) using R statistical software. Treatment means were separated using Least Significant Differences (LSDs) at 5% probability level. The multiplicative effects of G x E interactions were assessed by principal component analysis (PCA1 and PCA2) using GGE bi-plot software and adopting the following formula as recommended by (Yan et al.,2000).

$$Yij = \mu + \beta j = \lambda 1 \xi i 1 \eta i j 1 + \lambda 2 \xi i 2 \eta i j 2 + \varepsilon ij$$

Where: Yij= the performance of genotype i in environment j, μ = the grand mean, βj= the main effect of environment j, λ_1=singular values (SV) for the first principal component, λ_2= singular values (SV) for the second principal component, ξ_{i1} = eigenvector of genotype i for (Principal Component 1) PC1, ξ_{i2}= eigenvector of genotype i for (Principal Component 2) PC2, η_{j1}= are eigenvectors of environment j for PCl, η_{j2} = are eigenvectors of environment j for PC2 and εij= is the residual associated with genotype i in environment.

IV. Results

a) Effect of different environments on tuber yields

The combined analysis of variance showed that there were significant differences at ($p \leq 0.05$) among the evaluated potato genotypes. The environments were significantly different as well as the interaction between the environments and the genotypes (Table 2).

Table 2: Analysis of Variance (ANOVA) for table and Processing types for 2015 and 2016 long rainy seasons

	Table			Processing		
Source of variation	D.F	M.S	F pr.	D.F	M.S	F pr.
Genotype (G)	26	177.16	<.001	10	128.7	<.001
Environment (E)	5	5178.47	<.001	5	2351.47	<.001
G x E	130	99.44	<.001	50	54.22	<.001
Residual	810	33		329	27.36	
LSD Genotype	2.754			**2.417**		

Significant at level of P ≤0.05

The Table type genotypes showed varied performance across the environments. The average tuber yields in the evaluation environments were significantly different except in Burnt forest and Molo where mean tuber yields were almost similar (Table 3). Genotypes, G5 (32.49 t ha-1) and G15 (32.96t ha-1) yielded higher than the check genotype G11 (24.48 t ha-1) in Burnt Forest. The highest yielding check was genotype, G20 with an overall mean yield of 23.65 t ha-1. The other two checks, G11 and G24 had mean yield of 23.65 t ha-1 and 21.11 t ha-1 respectively. Genotypes, G4 and G7 performed better than G20 and G24 the commonly grown varieties in Burnt Forest. Table type genotype G21 with a yield range of between 12.86 and 24.55 t ha-1 was the lowest yielding. The environment with the highest tuber yield was Narok with a mean tuber yield of 29.26 t ha-1 whereas Kibirichia was the lowest tuber yielding location, with 12.79 t ha-1 mean yield. The highest yielding genotype was, G25 (20.15 t ha-1) in Kbirichia whereas, genotype, G9 with 7.72 ha-1 was the lowest yielding.

Table 3: Average tuber yields (yield t ha-1) of Table genotypes among test environments during long rainy seasons of 2015 and 2016

Genotype	Sites						
	B.Forest	Cherengany	Kibirichia	Molo	Narok	Timau	Mean
G5	32.49	34.59	12.61	24.1	27.05	28.08	26.49
G15	32.96	26.49	15.85	19.6	25.56	23.52	24.00
G23	16.34	13.57	16.99	19.82	31.89	27.77	21.06
G25	16.84	18.26	20.15	20.47	26.04	22.52	20.71
G19	13.85	14.34	16.78	19.81	32.8	26.39	20.66
G7	20.98	11.36	11.67	16.42	36.66	26.17	20.54
G4	20.78	19.13	12.3	17.79	28.19	22.88	20.18
G6	17.22	24.33	7.83	19.32	25.67	24.35	19.79
G18	19.11	15.55	10.81	20.30	30.67	22.00	19.74
G16	26.97	12.40	9.84	16.18	25.57	25.12	19.35
G27	15.54	12.72	15.13	20.95	27.12	23.67	19.19
G9	17.95	8.99	7.72	20.69	32.34	27.22	19.15
G21	12.9	12.86	13.29	23.93	24.55	18.92	17.74
Checks							
G20	18.34	27.76	13.15	21.05	32.82	28.75	23.65
G11	24.48	22.98	9.72	16.97	33.19	28.22	22.59
G24	14.35	26.61	10.77	24.56	27.98	22.41	21.11
Mean	20.07	18.87	12.79	20.12	29.26	24.87	21.00

Among the Processing types, G2 (29.1 t ha^{-1}) and G22 (20.1 t ha^{-1}) produced the highest yields in Burnt Forest (Table 4). Genotypes, G13 (17.14 t ha^{-1}) and G8 (17.72 t ha^{-1}) in Kibirichia had almost similar tuber yields. Genotype, G8, the commonly grown check gave similar yields to that of G13 (32.62 t ha^{-1}) in Narok. In Timau, the test genotypes had lower yields compared to G8, the check, though these yields were not significantly different. All environments were significantly different from each other for tuber yield. Narok with a mean yield of 29.26 t ha^{-1} was the highest yielding location, whereas, Kibirichia with a mean yield of 13.54 t ha^{-1} had the lowest tuber yields. Cherengany and Kibirichia were the lowest yielding locations with mean yields of 13.73 t ha^{-1} and 13.54 t ha^{-1} respectively.

Table 4: Average tuber yields of Processing genotypes among test environments during the long rainy seasons of 2015 and 2016

YIELD T HA^{-1}							
Gen	**B. forest**	**Cherengany**	**Kibirichia**	**Molo**	**Narok**	**Timau**	**Mean**
G2	29.07	15.75	11.88	19.67	30.43	24.32	21.85
G22	20.08	14.73	13.58	18.5	30.76	27.54	20.87
G13	13.91	11.93	17.14	20.52	32.62	24.31	20.07
G10	16.67	15.00	15.15	19.75	30.91	22.43	19.99
G26	16.56	17.52	12.66	22.3	24.93	24.37	19.72
G3	18.27	11.01	11.47	19.51	27.43	27.37	19.18
G17	21.38	10.76	12.78	18.51	23.23	26.4	18.84
G1	12.3	14.85	13.21	21.01	25.74	23.56	18.45
G12	15.12	14.73	11.22	15.8	25.63	20.81	17.22
G14	8.88	9.66	12.17	12.19	26.33	21.16	15.07
Check							
G8	11.54	15.14	17.72	22.07	31.61	29.04	21.19
MEAN	**16.71**	**13.73**	**13.54**	**19.08**	**28.15**	**24.66**	**19.31**

Adaptability and stability of potato Processing genotypes

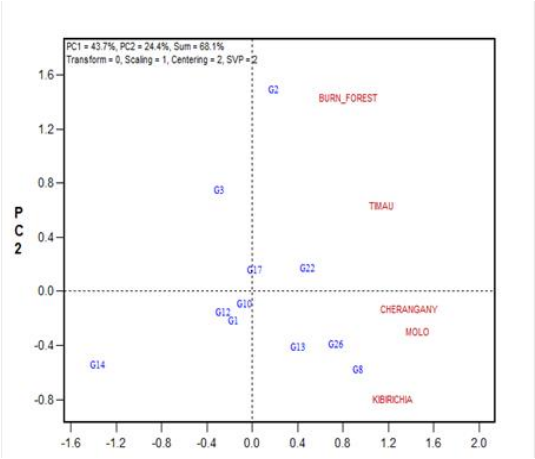

Figure 2: GGE-biplot showing the relative performance of Processing potato genotypes in Burnt forest, Timau, Cherangany, Molo and Kibirichia 2015 and 2016

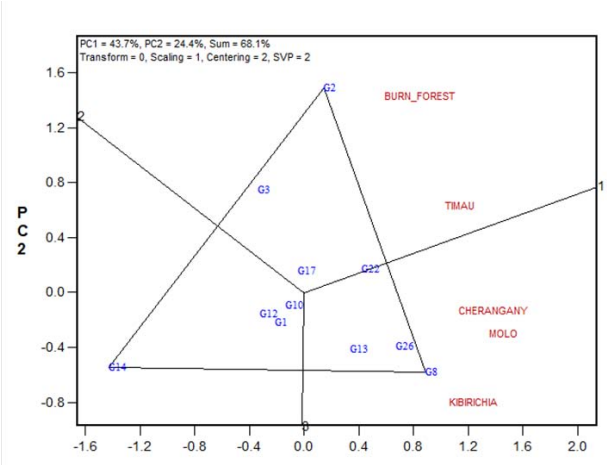

Figure 3: GGE Bi-plot analyses showing the mega-environments and the winning Processing genotypes during 2015 and 2016

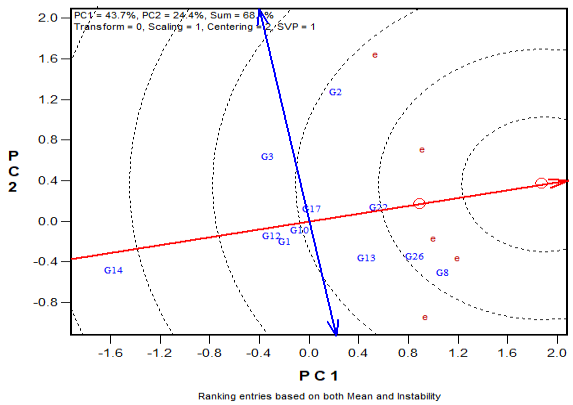

Figure 4: Positioning Processing types of genotypes relative to the ideal environment and their stability 'e' showing the distribution of environments during 2015 and 2016

The GGE bi-plot identified Burnt Forest and Timau environments as having both positive values for PC1 and PC2 (Fig 2). The Processing types, G2 and G22, gave positive values of PC1 and PC2. The two genotypes, G2 and G22, were specifically adapted to these two environments. Cherangany Molo and Kibirichia locations had large negative PC2 values which implied that they strongly interacted with the potato genotypes that had negative PC2 values. The genotypes in this region were adapted to the Cherengany, Molo and Kibirichia environments (Fig 2). Genotypes, G13, G26 and G8 were adapted to the Cherangany, Molo and Kibirichia environments. The PC1 and PC2 accounted for 43.7% and 24.4% of the variations respectively and together they accounted for 68.1% of the observed variations. In Fig 4, the double arrowed line that is perpendicular to the Average Environmental Coordinate (AEC), represents the genotypic stability and those genotypes on either side and far from it represent greater interaction with the environment and low stability whereas those closer are stable ones. The AEC points towards ideal genotype and ideal environment. The ideal genotype is one with higher mean and closer to the ideal environment represented by the small circle on the AEC. PC1 was associated with yield potential of the genotypes whereas PC2 was associated with the stability. G22 was identified as the most stable whereas G2 was the most unstable.

Two mega-environments were identified for the Processing types. The first one was Burnt Forest and Timau where, genotype, G2 was the highest yielding followed by genotypes, G3, G22 and G17 (Fig 3). The second mega-environment consisted of Cherengany, Molo and Kibirichia locations in which G8 yielded highest followed closely by G26 and G13. Genotypes G1, G10, G12 and G14 displayed low yields. The genotypes located to the right side of the polygon in Fig 4 were the high yielding ones whereas those to the left of the double arrowed line were the low yielding ones.

The midpoints of the concentric circles represent the position of an ideal genotype that is the most stable genotype with high mean tuber yield. The genotype that has the highest yield and is the most stable, shows the longest horizontal vector and shortest vertical vector (Bai et al. 2014). In Fig 4, the genotypes located closer to the ideal environment position were the highest yielding genotypes. Genotypes, G8 and G26 were closer to the ideal environment, whereas, G14 was furthest from the ideal environment and had the lowest yield. Genotypes, G8 and G2 gave high yields in specific environments but had low adaptability. Genotype, G22 was more stable than the check genotype, G8 even though the later was located closer to the ideal area. Genotype, G17 gave low yields but had higher stability compared to the check.

Adaptability and stability of potato Table genotypes

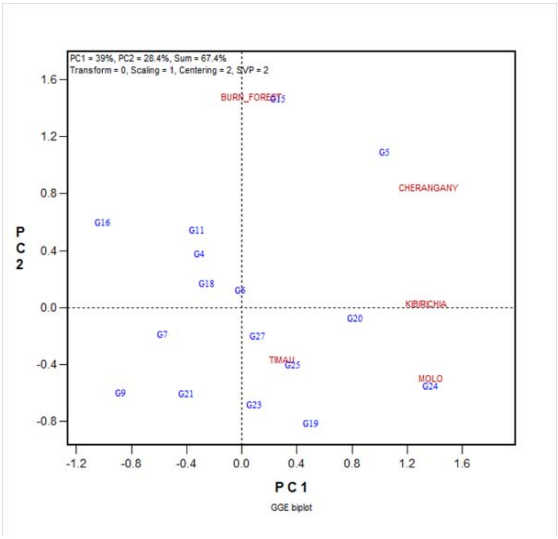

Figure 5: GGE-bi-plot showing general Table type genotypes yield performance relative to the test environments in 2015 and 2016 long rainy seasons

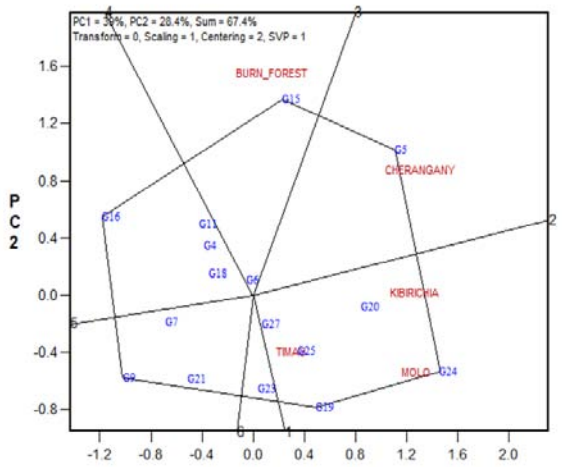

Figure 6: GGE-Biplot showing Table type yields and how they performed in different testing mega-environments during 2015 and 2016 long rainy seasons

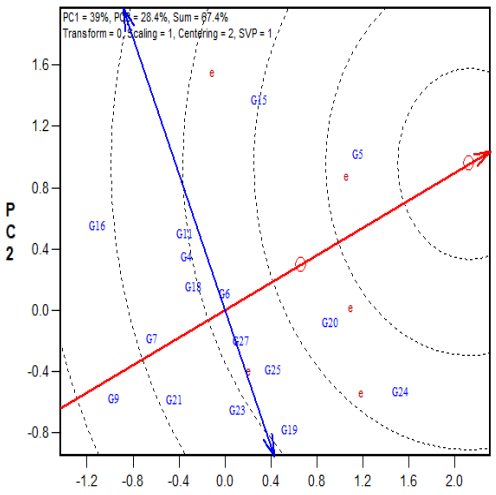

Figure 7: Stability ranking of the Table type genotypes relative to the ideal environment during 2015 and 2016 long rainy seasons

The GGE Biplot for Table types showed that PC1and PC2 explained 39% and 28.4% of the variations observed respectively and collectively explained 67.4% variation (Fig 5). Burnt Forest, Cherengany and Kibirichia locations had positive PC2 values, with G5 and G15 showing specific adaptability to Burnt Forest and Cherengany environments respectively. Timau and Molo locations had higher interactions with the genotypic effect and gave similar PC2 value to genotypes, G13, G19, G20, G23, G24, G25 and G27. Genotypes, G15 and G5 yielded highest in Cherengany whereas genotype, G9 and G21 had the poor yields. Genotypes, G27, G23, G20, G25 and G24 yielded better in Timau and Molo whereas genotypes G4, G7, G11, G9, G16, G18 and G21 were poor performers. Genotypes that had PC1 scores of >0 were high yielding and adapted to the production environment than those that had PC1 scores of <0 being poor yielders and were not adapted. The genotypes whose PC2 value was closer to zero were considered stable such as, G6 and those that had PC2 value far from zero such as G24 were considered unstable. Genotypes, G24 and G25 showed high adaptability in Molo and Timau environments respectively.

In Fig 6., the polygon graphs generated from GGE software showed the existence of three mega-environments among the evaluated locations for Table types. Burnt Forest, and Cherengany each formed one single mega-environment whereas Kibirichia, Timau and Molo formed the third mega-environment. The Table genotypes had varied levels of interaction with the environment (Fig 6. and Table 6.) with genotypes, G5, G9, G15, G16, G19 and G24 being positioned to the outermost corners of the polygon. These genotypes gave the higher yields in the mega environments whereas genotypes, G4, G7, G9, G11, G16, and G18

located in the interior of the polygon gave lower yields. On 'which won where', genotype, G15 won in Burnt Forest, whereas, G5 won in Cherengany and G24, in Timau, Molo and Kibirichia. PC1 and PC2 were accountable for 39% and 28.4% of the variation respectively.

Fig 7, shows that genotypes, G6 and G7 were more stable although they were low yielding compared to G5, G20 and G24 (Fig 7). G5, genotype gave the highest yields and was the most stable. Genotype, G15 even though was one of the high yielding ones, was unstable but had specific adaptation to Burnt Forest environment. Genotypes, G6, G19, G25 and G27 had their yields very close to the grand mean whereas, genotypes G4, G7, G9, G11, G16, G18 though low yielding were fairly stable.

Fig 8, shows the average tube yield of the Table and Processing genotypes variation among the environments. The yield between the two types ranged between 0.2 – 5.14 t ha^{-1} but for the environments, Cherengany had the highest yield difference at 5.14 t ha^{-1} and Timau had the lowest at 0.21 t ha^{-1} (Fig.8). The Processing genotypes were more sensitive to environmental factors than the Table types. This was most experienced in Cherengany and Burnt Forest environments as reflected by their yield differences.

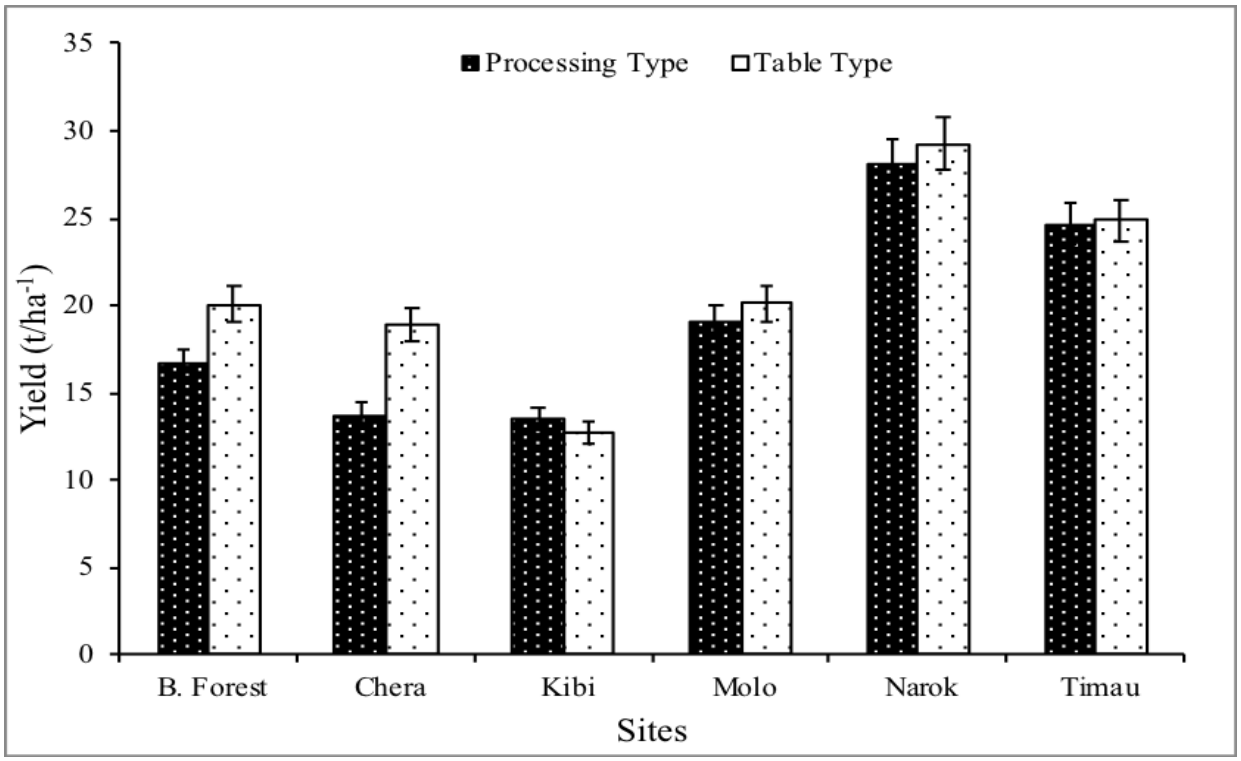

Figure 8: Comparison between Table and Processing types based on means from each environment during long rainy seasons of 2015 and 2016

V. Discussion

Analysis of variance for tuber yield revealed diverse and highly significant genotype by environment interactions (G x E). The significant G x E interaction is as a result of variations in tuber yield that was associated to the different sensitivity levels of the genotypes to environmental conditions. This was attributed to the extensive genetic variation that exists within and between the elite potato genotypes that control to tuber yield and the differences in environmental factors that influence tuber yield (Suttle, 2007; Jyotshnarani et al., 2017, Brandon et al., 2019). The significant mean sum of square of G×E interaction for tuber yield showed that the genotype response varied in different environments (Jyotshnarani et al., 2017) and the responses were due to the diverse genetic constitution. Factors within the different environments that are both predictable and un-predictable were responsible for the yield variations (Karimizadeh et al., 2012). Every genotype responded differently depending on its sensitivity levels.

The diversity in yields within and between the types also demonstrated that the potato factors controlling tuber yield responded differently to different external factors presented by the different environments (Tables 3.and 4). Some genotypes maintained stable yields while others had major yield fluctuations. For example, G22 and G6 for Table and Processing respectively were stable, whereas G15 and G2 were the most unstable. Genotype, G15 of the Table type had a superior mean yield across environments compared to

the other test genotypes and the checks but was unstable compared to the low yielding but stable, G6 genotype (Fig.7). Similar variations were observed among the Processing genotypes with G2 having high yields but was unstable whereas G22 was stable but gave lower yields. Some stable genotypes such as G6 and G17 had low yields while some unstable genotypes such as G2 and G15 had higher yields in the two types. Therefore, stability of a genotype does not necessarily lead to a high yield performance of a genotype. This indicates that some genotypes were genetically better buffered compared to those that had varying responses to environmental conditions (Jyotshnarani et al., (2017); Haydar et al., (2009). The average tuber yields the of genotypes across environments ranged between 29.26 t ha-1 in Narok to 12.79 t ha-1 in Kibirichia for the Table types whereas for the Processing types, the yields ranged between 28.15 t ha-1in Narok to13.54 ha-1 for Kibirichia (Tables 3 and 4) indicating that Narok had the best conditions for tuber yield for both types whereas Kibirichia had the most tuber yield stressing factors.

There were no noticeable variations due to seasons meaning that genotypes were not sensitive to seasonal variations. This indicates that the seasonal changes were not determining genotypic response. The stable genotypes were not necessarily the high yielding ones within the mega environment or the micro environments. Taking this into account, genotypes such as G5 and G15 for the Table potato type and G22 for the Processing type should be selected for tuber high yield.

The two potato types had low mean yields in Kibirichia, Narok location recorded the highest yield for the two types (Tables 3,4. and Fig 7). This shows that, Narok has most of the required potato production nutrients and the cool and humid weather conditions that are conducive for potato production. The two potato types had some specific requirements that are necessary for optimum yield, as shown by the variation in yield of each type from one environment to the next (Fig 8). Where these resources are limited and or not readily accessible in optimum quantities when needed, the affected genotype performs poorly. The selection of adapted genotype faces many challenges when based on environmental means rather that genotype mean.

The micro-environments that form a mega-environment have a lot of similarities than differences and therefore genotypes are subjected to almost similar conditions in any of the microenvironment. Two mega-environments for Processing type and three mega-environments for Table type were identified by GGE biplots (Fig. 3 and 6) respectively. The formation of different number mega-environment formed is an indicator of some differences in genetic responses to environmental pressures. The mega-environments provide a guide on judicious utilization of resources without compromising on quality of the recommendations and decisions that can be derived from collected data (Affleck., 2008). Timau, Molo, Kibirichia environments had similar characteristics and therefore any two the sites could be eliminated during varietal evaluation and still provide reliable and representative information for the Table genotypes. Cherengany and Burnt Forest environments, came out as different independent mega-environments meaning that varietal evaluation could be conducted in any one of these environments. Similarly, for the Processing types, either Burnt forest or Timau environment could and still dependable data obtained.

The mega-environments that were obtained for both the Table and Processing potato genotypes, showed that some locations were similar while others were different. Tuber yield and stability of the test genotypes are important aspects to determine the suitability of a genotype for recommendation in a particular location. For the Processing types, genotype, G22 demonstrated the highest stability whereas genotype G2 was the most unstable (Fig 4). Among the Table types G5 was the highest yielding genotype that was also fairly stable and was closer to the ideal environment, whereas G19 was the most unstable (Fig 7). In both Table and Processing types, the stable genotypes had fairly consistent yields across contrasting environment compared to the unstable ones that had low yields (Table 3 and 4). The stable genotypes were those that had insignificant interaction with the environment whereas the unstable ones were those that significantly interacted with the environment (Bogdan et al., 2014).

There was expression of both general and specific adaptability among the genotypes used in the study. For example, among the Table type genotypes, G5 and G15 expressed general adaptability by giving consistently high yield across environments whereas the other genotypes only displayed good performance in specific environments (Table 3 and Fig 7). In both potato types evaluated here, there were those that had dismal performance across all the experimental sites. For example, the Processing type had their poor performers as G1, G12 and G14 and the Table type had, G21, G9 and G27. The best performing genotypes passed as the best adapted genotypes because of their dependable tuber yield. For crop improvement reasons, the genotypes with a combination of high mean tuber yield and high stability are possibly the reliable genotypes for selection and further evaluation (Bai et al.,2014) though this situation is a rare combination.

The genotypes of the two potato types, Table and Processing, expressed both static and dynamic stability. Static stability was expressed by some genotypes that yielded in a fairly similar manner in more than one site. For example, G6 and G22 of the Table type and Processing respectively expressed static form of stability whereas dynamic stability was expressed by

genotypes G20 and G26 Table and Processing respectively. In these cases, the yields of the genotypes varied significantly from one location to another but did not differ significantly from the environmental mean. The Table type genotypes, namely, G6 and G7 showed static stability, an indication that the genetic strength of these genotypes had been stretched to the maximum and no agronomic improvement with favourable climatic conditions could alter their performance significantly. The Processing genotypes, G22 and G17 expressed static stability whereas G2, G13 and G26 and the check G8 showed dynamic stability.

From the results, it was clear that the sensitivity of genotypes to environment differed among and within the Table and Processing types. Some environments produced almost similar yield and therefore could be classified as being related. Positively correlated environments have similar conditions hence similar discrimination while those that are not related present unrelated yields (Yan and Hunt 2001). This means, one of the environments could be eliminated and still reliable data obtained in the future evaluations (Bai et al., 2014). Burnt Forest, Kibirichia and Molo were the most discriminating environments. Varietal evaluation could be conducted in any one of the three environments to save the time and resources.

The Processing types were the most susceptible to the negative effects of the environment compared to the Table types. Bernie et al., (2011) associated the variation of genotype performance to differential gene expression in response to different environmental conditions. Tumwegamire et al. (2016) recommended that stability and adaptability studies be carried out on new genotypes before deployment to determine the potential of the genotypes. This study showed that there were differences in adaptability and stability within and between the potato types in the different environments. Genotypes that were less sensitive to secondary effects were the most stable compared to those that were sensitive to secondary effects (Gehan et al., 2015).

VI. Conclusion

Genotypes, G5 and G22 were the highest yielding among the Processing and Table types respectively. Genotypes, G2, G8, G13, G17 and G26 gave high yields with relatively low stability, whereas, genotypes G3, G10, G12 and G14 were unstable. The unstable genotypes, with low yields may qualify as being adapted to specific environments. Narok and Timau environments provided favourable conditions for both Processing and Table genotypes. Kibirichia was the least favourable environment whereas, Narok was the most favourable environment for potato production. G x E interactions significantly affected the yielding ability of all genotypes and therefore, their effects should be determined before deploying new varieties to target environments. G x E interactions for other traits need also be analyzed to establish their stability before deployment.

Acknowledgements

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Genetic Diversity and Population Structure Analysis of Tropical Soybean (*Glycine Max* (L.) Merrill) using single Nucleotide Polymorphic Markers

By Tonny Obua, Julius P. Sserumaga, Stephen O. Opiyo, Phinehas Tukamuhabwa, Thomas L. Odong, Josiah Mutuku & Nasser Yao

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Abstract- Soybean (Glycine max (L.) Merrill) is among the most important crops worldwide due to its numerous uses in feed, food, biofuel, and significant atmospheric nitrogen fixation capability. To understand the genetic diversity and population structure of tropical soybean germplasm, 89 genotypes from diverse sources were analyzed using 7,962 SNP markers. The AMOVA results showed low diversity among and high within the populations, while the polymorphism information content (PIC) was 0.27. Both phylogenetic and principal component analysis grouped the 89 soybean genotypes into three major clusters, while population structure grouped the soybean genotypes into two subpopulations. On the other, the average Roger genetic distances within the study population was 0.34. The low diversity reported in the studied soybean germplasm pool is particularly worrying, considering the new trends of climate change and the emergence of new pests and diseases of soybean. Therefore, in order to address these challenges and develop soybean varieties with desirable traits, there is a need to broaden the genetic base of tropical soybean through the importation of germplasm from other countries.

Keywords: genetic diversity, population structure, single nucleotide polymorphism (SNP), soybean, tropical soybean genotypes.

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I. Introduction

Soybean is among the most valuable crops in Uganda and across the East African region due to the high protein content that makes it an important ingredient in the diets of the people and livestock (Tukamuhabwa, 2001). Several soybean processing plants have been established in Uganda and across the East African region with large processing capacities to develop different products from soybean. This new development has motivated the farmers to produce more grains to supply these plants (Tukamuhabwa et al., 2019). The three leading African countries in soybean production are South Africa (1,540,000 MT), Nigeria (758,033 MT), and Zambia (302,720 MT) (FAO 2018). Uganda is 11th in Africa and 1st in East Africa, with a production of 29,000 MT(FAO 2018). Hence soybean production and consumption have led to increased farmers' income, improved food and nutrition security, and poverty eradication at the rural household level (Ssengendo et al., 2010; SNV, 2011; Tukamuhabwa & Obua, 2015). Accordingly, soybean has the potential to contribute to poverty alleviation in Uganda and across the East African region.

Despite the contribution of soybean to smallholder farmers in Uganda and across the East African region, development of new varieties has been hindered by the low genetic diversity of the crop that have been observed in other countries (Gupta & Manjaya, 2017; Kumawat et al., 2015; Liu et al., 2017; Maldonado dos Santos et al., 2016; Torres et al., 2015). Kumawat et al. (2015) investigated the diversity of 82 Indian soybean accessions using SSR markers and identified three major clusters. In another study, Torres et al. (2015) found that both Principal Component Analysis (PCA) and STRUCTURE, clustered 191 soybean accessions in Brazil into two groups. Similarly, Gwinner et al. (2017) in another study that aimed at understanding the genetic diversity and population structure of 77 commercial soybean varieties in Brazil using 35 SSR markers, reported low genetic diversity in soybean germplasm.

To assess the genetic diversity of soybean and other plants, various methods such as morphological markers, geographic origins, pedigree information, isozymes, and DNA markers have been applied (Dayaman, 2007; Appiah-Kubi, 2012; Ojo et al., 2012; Malek et al., 2014; Villela et al., 2014). The use of morphological trait has remained a powerful taxonomic tool for preliminary grouping of germplasm before their classification using more precise marker techniques.

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Infact several studies involving the classification of plants still rely on the use of morphological traits (Khalid et al., 2010). Additionally, the use of morphological markers in classification is easy to score, cheaper and fast. However, the disadvantage of using morphological markers is that it's less robust compared to most molecular markers and outcomes can be easily influenced by environmental factors. In the case of pedigree information, limitations such as uncertain and incomplete data errors are likely, while for isozymes, chances of limited data are more prominent (Li & Nelson, 2001; Wang et al., 2006). So far, DNA markers remain the most precise method of genetic diversity analysis that have been complemented with morphological trait analysis. Among different DNA markers, random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) have been widely used in understanding the diversity of soybean; each with its advantages and disadvantages (Chauhan et al., 2015; Chen et al., 2017; Doldi et al., 1997; Hipparagi et al., 2017; Ojo et al., 2012; Ren et al., 2013; Singh et al., 2013; Tantasawat et al., 2011; Tanya et al., 2001; Torres et al., 2015). SSR markers have been widely used to determine genetic diversity in many crops because they are easy to use, reasonably low price, and high level of polymorphism (Vignal et al., 2002). However, recently SNP markers have been widely utilized for assessment of diversity in plants because they occur much more frequently in the genome than SSR markers, and their genotyping can be easily automated (Mammadov et al., 2012). In the current study, we used Genotype By Sequencing (GBS) technology to study a collection of 89 tropical soybean germplasm collected from different countries. Therefore, the objective of the current study was to understand the genetic diversity and population structure of tropical soybean germplasm using SNP markers. Since the genotypes included in the current study are parental lines, land races, released varieties, and advanced lines, they are representative of the existing germplasm in tropical Africa.

II. Materials and Methods

a) Plant materials

In this study, we used a total of 89 tropical soybean genotypes; these included collections from different sources that possess high genetic diversity (45 genotypes were from Uganda, 13 from Japan, six from the USA, 12 from World Vegetable Center (AVRDC) in Taiwan and 13 from Seed Co; a seed Company from Zimbabwe (Supplementary table).

b) DNA extraction, Determination of DNA Quality and Quantity

Seeds from each genotype were grown under controlled greenhouse conditions at Biosciences eastern and central Africa - International Livestock Research Institute (BecA - ILRI) Hub, Kenya. Twelve days after germination, one young leaf from one plant from each genotype was harvested, and DNA extracted using ZR Plant / Seed DNA Mini Prep™ according to manufacturer's protocol with minor modifications.

The DNA quality was first checked on 0.8% (w/v) agarose gel in 1 X Tris-acetate EDTA buffer and ran at 80V for 45 Minutes. The run gels were photographed using GelDoc-It™ Imager(UVP) and the picture image interpreted for DNA quality. The DNA was quantified using Thermo Scientific Nanodrop2000C Spectrophotometer and stored at 4 °C.

c) SNP Genotyping

The soybean genotypes were genotyped using the Illumina HiSeq 2500. Genotyping was conducted at Diversity Arrays Technology (DArTSeq™) in Australia. The genotypic process of the samples followed an integrated DAr T and genotyping-by-sequencing (GBS) methodology that involved complexity reduction of the genomic DNA, and repetitive sequences were eliminated using methylation-sensitive restrictive enzymes before sequencing on next-generation sequencing platforms (Kilian et al., 2012).The soybean reference genome was downloaded from ftp://ftp.jgi-psf.org/pub/JGI_data/phytozome/v7.0/Gmax. The sequence data generated were then aligned to the soybean reference genome sequence, Soybean_v7, to identify single nucleotide polymorphisms (SNPs) markers.

d) Data analysis

GBS data from a total of 16,688 SNPs, distributed across all the 20 soybean chromosomes was received from Diversity Arrays Technology (DArTSeq™), Australia. The genotype data was filtered using a minor allele frequency (MAF) of 0.05 and a minimum count of 80% of the sample size using TASSEL v.5.2.43 software (Bradbury et al., 2007). Genetic distance was calculated between a pair of inbred lines in dataset using the identity by state similarity (IBS) method implemented in TASSELv.5.2.43. A marker-based kinship matrix was then calculated between a pair of inbred lines in data set using TASSELv.5.2.43.

Population structure was estimated using the model-based clustering approach implemented in STRUCTURE v2.3.4 software (Pritchard, Stephens & Donnelly, 2000). To estimate the posterior probabilities (qK) a 100,000 burn-in period was used, followed by 100,000 iterations; with the hypothetical number of subpopulations (k) ranging from 1 to 10, with ten replicates for each K. The number of subpopulations was determined when Δk reached its highest value (Evanno, Regnaut & Goudet, 2005). The Delta K was

calculated for each value of K using Structure Harvester (Earl, Cruz, and Vonholdt 2012; Evanno et al. 2005). A line was assigned to a given cluster when the proportion of its genome in the cluster (qK) was higher than a standard threshold value of 70 %. For the chosen optima value of K, membership coefficient matrices of replicates from STRUCTURE were integrated to generate a Q matrix using the software CLUMPP (Jakobsson and Rosenberg, 2007) and the STRUCTURE bar plot was drawn using the DISTRUCT software (Rosenberg, 2004). Principal coordinate analysis was performed based on the genetic distance matrix using the Dissimilarity Analysis and Representation for windows (DARwin) v.6.0.013 (http://darwin.cirad.fr). To validate and gain more insight into the genetic diversity of the soybean germplasm panel used in this study, we generated a phylogenetic tree by the neighbor-joining method. Analysis of molecular variance (AMOVA) was performed using GenAlEx V6.5 software.

III. Results

a) Genotype Diversity analysis

A total of 16,688 SNP markers were identified in the 89 genotypes of soybean; of those 7,962 polymorphic and non-redundant SNP markers, with greater than 5% minor allele frequency (MAF) and missing data lower than 20% were used for subsequent analysis. These 7,962 SNPs detected a total of 15,924 alleles as expected. The average PIC was 0.27, ranging from 0.01 to 0.50, and heterozygosity ranged from 0.0 to 0.35 of individuals and 0.0 to 0.8 of markers (Fig. 1).

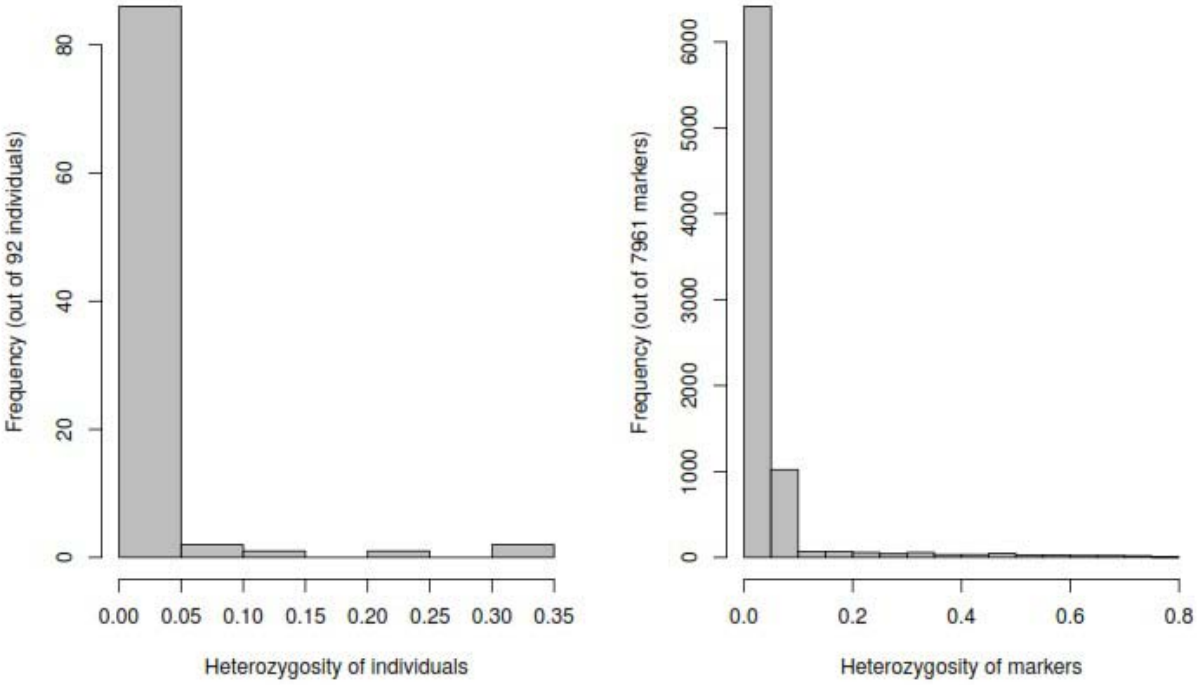

Fig. 1: Levels of heterozygosity of individual soybean genotypes and SNPs markers

b) Genetic distance and relationship

The average Roger genetic distances within the study population was 0.34. From a total of 89 genotypes, 18.1% of the distance values were between 0.0 and 0.05, while 20.7% were between 0.35 and 0.40 (Fig. 2). Relative kinship reflects the approximate degree of identity between two given genotypes. For combined analysis of all 89 genotypes, the kinship coefficients ranged from 0 to 1.04, with an overall average of 0.51; only 1.6% of the pair wise kinship estimates had values of 0.0 – 0.05 while 76.1% had values ranging from 0.5 – 0.550, indicating that most of the genotypes were in one way or another related and very few genotypes were not related (Fig. 3).

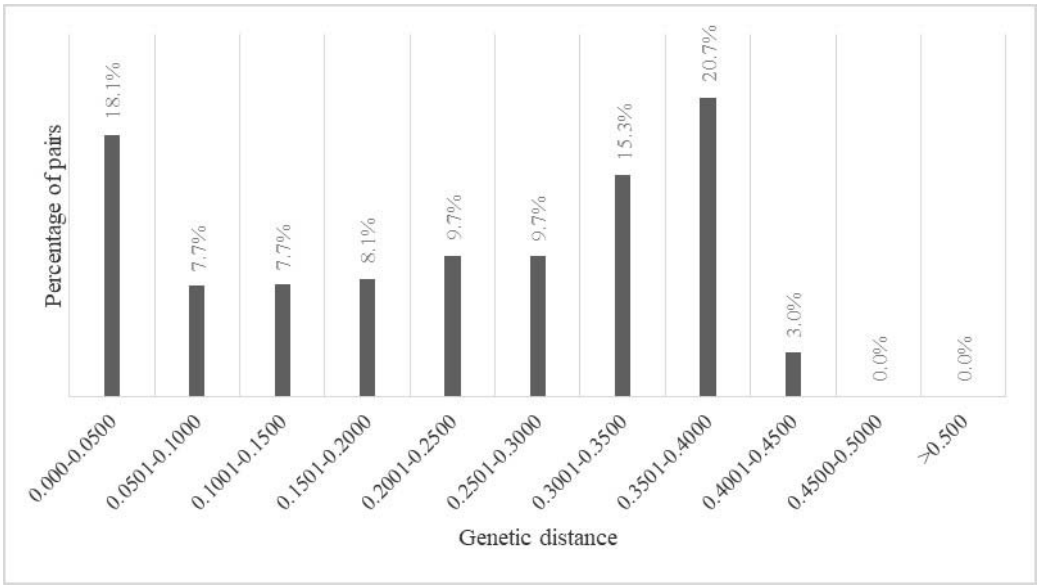

Fig. 2: Distribution of pairwise Roger's genetic distance calculated for 89 soybean genotypes

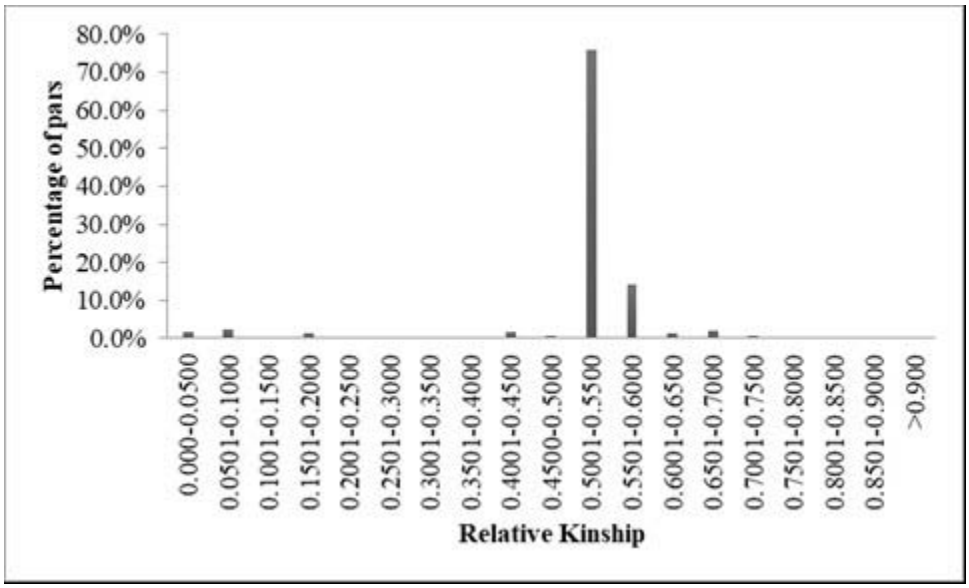

Fig. 3: Distribution of pair-wise kinship coefficients among 89 soybean genotypes from different sources based on 7,692SNPs

c) Population structure analysis

The log probability of the data LnP (D) increased continuously with increasing K (number of groups or populations). The ad hoc statistic ΔK showed a higher likelihood value at K = 2 as the highest level of structure (Fig. 4). This pattern was also observed in the population structure, where two groups were formed (Fig. 5).

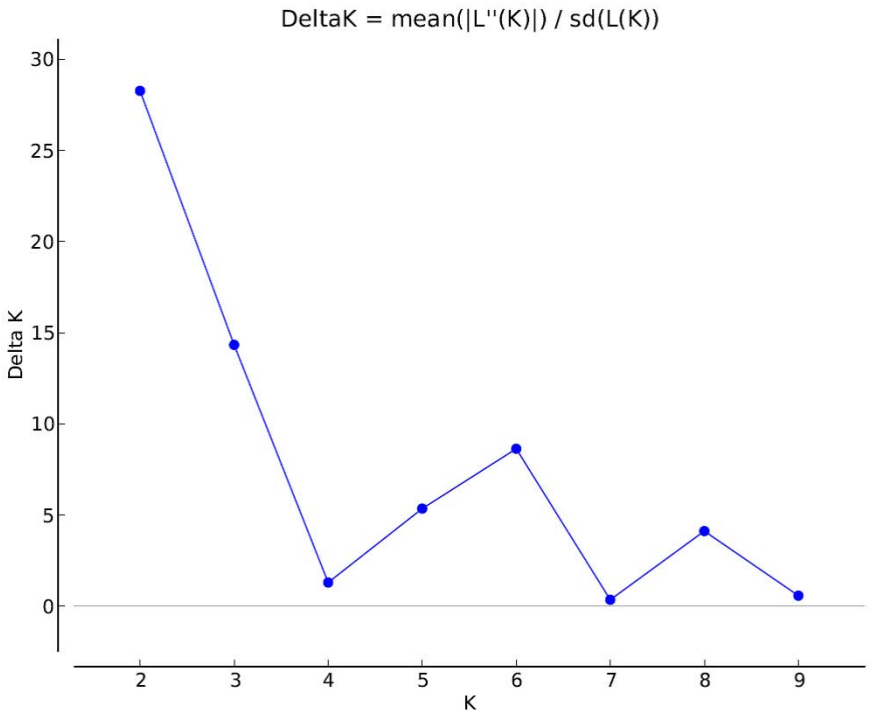

Fig. 4: Estimation of population using LnP(D) derived ΔK with K ranged from 1 to 10 with 7,692SNPs

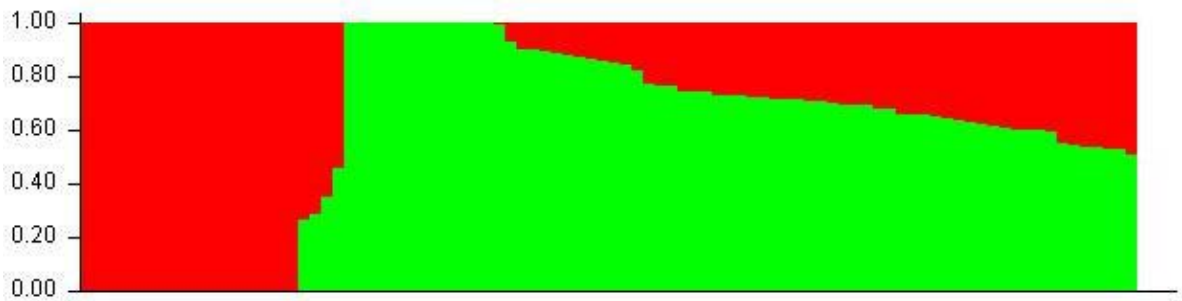

Fig. 5: Population structure (K = 2) inferred from STRUCTURE analysis for the 89 soybean genotypes based on 7,692SNPs

d) Neighbor-joining Phylogenetic Tree

The phylogenetic tree grouped the 89 soybean genotypes into three major clusters (Fig 6). The genotypes were separated into three distinct sub-clusters: There were 40 genotypes in sub-cluster 1, which included Nam II and GC00138-29, and 13 progenies derived from a cross between these two genotypes. Nam II is a Ugandan variety, which is a selection from TGM 79; obtained from IITA while GC00138-29 is a variety from AVRDC in Taiwan. This sub-cluster also included released varieties in Uganda; Namsoy 3 which is a cross between Kabanyolo 1 and Nam 1 (selection from ICAL 131 from the USA), and Maksoy 5N that is a progeny of Nam II and GC00138-29. The second sub-cluster had 26 genotypes, among which 13 genotypes were from Seed co in southern Africa and eight genotypes from AVRDC, Taiwan. It was surprising that Namsoy 4M, a released Ugandan variety that is a progeny of Nam II and GC00138-29, was clustered in this sub-cluster. By comparison, the other remaining 23 genotypes belonged to sub-cluster 3, among which seven genotypes were progenies from a cross between Duiker and TGx 1835-10E while nine were from a cross between Duiker and GC00138-29. This sub-cluster also included released Ugandan varieties, Maksoy 1N (selection from TGx 1835-10E), Maksoy 2N (Duiker X TGx 1835-10E), Maksoy 3N and Maksoy 4N (Duiker X GC00138-29). However, few S lines and AVRDC genotypes were scattered in all three major clusters.

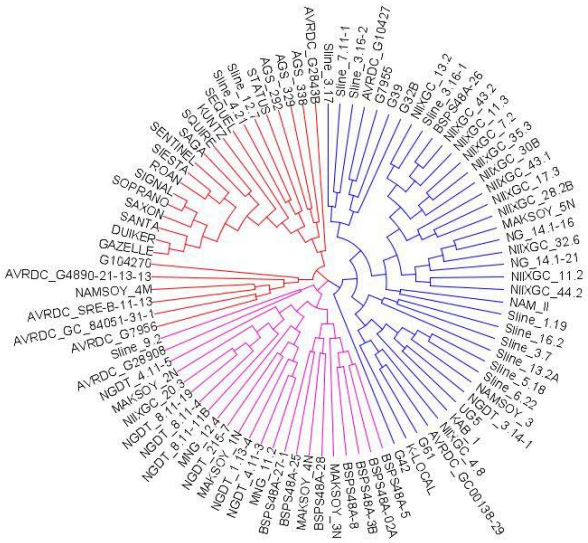

Fig. 6: Tree based on the *Neighbor Joining* method showing genetic dissimilarity between soybean genotypes, based on SNP markers

e) Principal Component Analysis (PCA)

PCA has been suggested as an alternative to population structure analysis for studying population stratification from genotypic data (Patterson et al., 2006). A PCA of the 89 genotypes with the 7,962 SNPs also showed a clear separation of the same three major groups that were identified by the phylogenetic tree (Fig. 7).

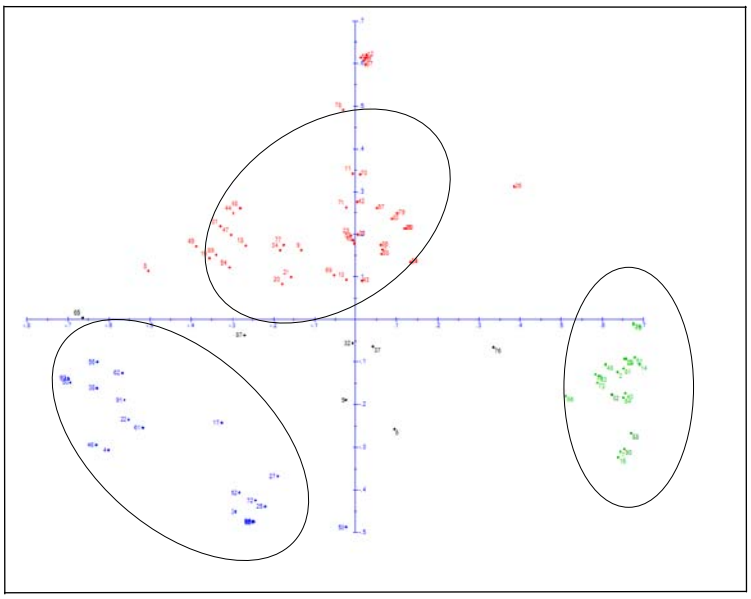

Fig. 7: Plot of PC1 (40.6%) and PC2 (18.2%) from principal coordinate analysis based on genetic distance matrix calculated for 89 soybean genotypes genotyped with 7,692SNPs

f) Analysis of molecular variance

Analysis of molecular variance (AMOVA) among the 89 soybean genotypes indicated that 2% of the variance was due to genetic differentiation among the populations, 98% of the variance was accounted for by genetic differentiation among individuals within populations.

IV. Discussion

One of the requirements for a successful breeding program is a high level of genetic diversity among the germplasm used for the development of new crop varieties. Over the years, most soybean breeding programs have replaced traditional varieties or

landraces with more modern varieties with desirable attributes that have led to increased yields. However, in the current study, to compare the genetic diversity of tropical soybean genotypes, we studied fairly diverse sets of genotypes from Uganda, Zimbabwe, Japan, Taiwan, and the USA. These genotypes included parental lines, land races, released varieties, and advanced lines that are representative of the existing germplasm in tropical Africa.

The level of genetic diversity observed in this study is lower compared to previously reported results based on SNP data (Li et al. 2010; Hao et al. 2012; Zhou et al. 2015). The observed low diversity is because the genotypes used in the present study were mainly released varieties and advanced breeding lines. In contrast, the genotypes used in Li et al. (2010), Hao et al. (2012) and Zhou et al. (2015) included mainly wild relatives and landraces of soybean. On the other hand, previous studies that involved improved soybean varieties also observed low genetic diversity (Liu et al. 2017; Maldonado dos Santos et al. 2016). These improved varieties tend to have low genetic diversity because of the high selection pressure subjected to the genotypes during evaluation and selection (Gwinner et al. 2017). This was also confirmed by genetic distance and kinship analysis that showed that majority of the genotypes in this study are related to each other in one way or another.

The phylogenetic tree and PCA analyses indicated the existence of three major sub-clusters among the 89 genotypes of our study. On the other hand, population structure clustered the genotypes into two major subpopulations. Sub-cluster 1 included Nam II and GC00138-29 and 13 progenies derived from a cross between these two genotypes. Nam II is a Ugandan variety, which is a selection from TGM 79; obtained from IITA while GC00138-29 is a variety from AVRDC in Taiwan. This subpopulation also included Maksoy 5N, released in 2013 and NII X GC 44.2 that was released in 2017 as Maksoy 6N and are progenies of Nam II and GC00138-29 cross. Since TMG 79 and GC00138-29 were introduced to Uganda through Consultative Group for International Agricultural Research (CGIAR) institutions that usually collect germplasm from different countries, there is a possibility that they share the same geographical origin. On the other hand, genotypes from Seed Co and AVRDC, Taiwan were grouped in the second sub-cluster. This implies that soybean varieties from Seed Co share very similar parents and geographical origin with genotypes from AVRDC.

By comparison, the third sub-cluster mainly consisted of progenies from two crosses; Duiker X TGx 1835-10E and Duiker X GC00138-29. The sub-cluster also included released Ugandan varieties; Maksoy 1N (selection from TGx 1835-10E), Maksoy 2N (Duiker X TGx 1835-10E), Maksoy 3N and Maksoy 4N (Duiker X GC00138-29). Duiker originated from Zimbabwe and was used as a female parent during generation of the two crosses.

V. Conclusion

Genetic variation and population structure of the core germplasm available for soybean breeding in Uganda and the East African region were assessed using high-density SNP markers. The results of the study showed a low level of heterogeneity within most of the genotypes studied, suggesting that the current generation of inbreeding has fixed lines. The observed low diversity in the germplasm pool is particularly worrying; considering the vulnerability of agriculture under the impact of climate changes. For example, in Uganda, we have observed the emergence of two new soybean pests (groundnut leaf miner and bruchids) that previously were not main production constraints. This is coupled with breakdown of soybean rust resistance in the existing soybean varieties in Uganda that were previously resistant to the disease due to several virulent races of soybean rust pathogen. Therefore addressing these challenges and developing soybean varieties with the desirable traits, requires diversification of the genetic background of the current breeding population by incorporating new genetic resources from other countries.

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Documentation of Indigenous and Introduced Soil and Water Conservation Practices in Southern Ethiopia

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Abstract- In Ethiopia soil erosion by water significantly contributes to food insecurity among rural households and poses a real threat to the sustainability of existing subsistence agriculture. In many parts of Ethiopia particularly southern Region many introduced and indigenous soil and water conservation practices were implemented using different approach. However, indigenous soil and water conservation practices adopted at farmers field, types, their names, technical dimension and their socio economic importance in the village is not well documented for further studies. Therefore, the present study was conducted in Gamo Gofa, Segen area peoples and Basketo special distict of the Sothern Ethiopia. As a methodology, discussion was organized at zonal and woreda level agricultural offices and sample of woredas and kebeles having similar farming system were selected with systematic sampling approach.

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Documentation of Indigenous and Introduced Soil and Water Conservation Practices in Southern Ethiopia

Birhanu Wolde [α] & Wudinesh Naba [σ]

Abstract- In Ethiopia soil erosion by water significantly contributes to food insecurity among rural households and poses a real threat to the sustainability of existing subsistence agriculture. In many parts of Ethiopia particularly southern Region many introduced and indigenous soil and water conservation practices were implemented using different approach. However, indigenous soil and water conservation practices adopted at farmers field, types, their names, technical dimension and their socio economic importance in the village is not well documented for further studies. Therefore, the present study was conducted in Gamo Gofa, Segen area peoples and Basketo special distict of the Sothern Ethiopia. As a methodology, discussion was organized at zonal and woreda level agricultural offices and sample of woredas and kebeles having similar farming system were selected with systematic sampling approach. Focus group discussion, questioner and transect walk were made in the selected kebele and the technical aspects of identified SWC practices were measured and described as well. Some of the most common identified indigenous and introduced conservation practices are, mulching, Intercropping, Trenches, cut of drain, grass integrated with soil ,stone terraces , Targa, pataya, korayida, Aflimayita fanyajju terraces, Agro-forestry practices like Home garden, live fence, park land agro forestry (combination of Moringa Stenophetala , Mangifera indica, Gravelia rebusta Terminalia browenii ,Cordia africana, banana, maize and other fruit tree species. Therefore, the identified practices provide information for researchers, extensions and other conserved body to do more in the area of soil and water conservation and should be proven in the research. It is better to conduct detail study and disseminate.

Keywords: indigenous, introduced, soil and water conservation practices.

I. Introduction

In Ethiopia soil erosion by water significantly contributes to food insecurity among rural households and poses a real threat to the sustainability of existing subsistence agriculture (Yirga, 2007). In response to this problem, soil and water conservation (SWC) activities were launched by government to implement physical and biological soil and water conservation measures by community collective action (mass movement). On the other way, different land enhancing technologies and practices have been introduced by research institutions, extension and other development practitioners in the region (Wagayehu and Lars, 2003).

Indigenous soil and water conservation is the method used different farmers to facilitate optimum level of production from a given area of land while keeping soil loss below a critical value. The soil loss tolerance value is defined as the rate of erosion at which soil fertility can be maintained over at least 25 years (Hurni, 1983). Indigenous soil and water conservation practices have very often been ignored or underestimated by development agents, researchers, soil conservationists and government staff (IFAD, 1992).

Although the objectives of knowing indigenous soil and water conservation practices give us an understanding of farmers' way of thinking about the measures (Hudson, 1992). Farmers use a number of indigenous soil and water conservation technologies to prevent the problem of soil erosion. Among these are cut- of -drains, leaving crop residues in the field, distribution of manure, contour farming, fallowing, planting root crops by preparing bunds, tree planting on slope farm, use of trash lines on contour, row planting, alley cropping, intercropping, strip planting, and plantation of Sisal (*Agave sisalana Perrine*) and euphorbia (*Euphorbia classenii*) on the farm etc. The indigenous soil conservation practice of Konso community is developed over a very long period of time. *(Yeshambel 2013)* UNESCO has registered the terraces of the Konso people of Southern Ethiopia as one of the world heritage (Shimelis, 2011). According to Genene M. and Abiy G. (2014), most of the farmers in south western Ethiopia practices introduced and indigenous soil and water conservation activities like; contour farming, furrow making, residue leaving, agronomic practices, putting trash lines on contour etc.

Broadly the conservation measures are classified as agronomic measures, physical /structural/ measures and biological/vegetative/ measures (IFAD, 1992). The definition of each broad type of ISWC practices is as follows:

Agronomic: These are measures undertaken within the cropping area for crop production purposes and include practices such as intercropping, contour cultivation, minimum tillage, mulching, manure etc. which:

- Are usually associated with annual crops

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- Are repeated routinely each season or in a rotational sequence
- Are of short duration and not permanent
- Do not lead to changes in slope profile
- Are not zoned
- Are independent of slope

Biological/Vegetative/: These measures involve the deliberate planting of trees, shrubs, grasses etc, or retention of areas of natural vegetation (eg. reforestation, contour hedgerows, and natural vegetative strips) which:

- Involve the use of perennial grasses/pasture legumes, shrubs or trees
- Are of long duration
- Often lead to a change in slope profile
- Are often zoned on the contour or at right angles to wind direction
- Are often spaced according to slope

Structural/ physical/: Measures which involve the construction of physical structures (e.g. graded banks or bunds, contour stone lines, level bench terraces, artificial waterways and drop structures) which:

- Lead to a change in slope profile
- Are of long duration or permanent
- Are carried out primarily to control runoff and erosion
- Require substantial inputs of labour or money when first installed
- Are zoned on the contour
- Are spaced according to slope

Appropriate soil and water conservation technologies are those which offer for a given production situation an optimal solution for using the land for sustainable and productive agricultural purposes. Appropriate technologies are not necessarily "simple" technologies. However, in the context of many developing countries, the appropriate technologies will be ones which are not capital-intensive and which use local resources and the existing labour force in an optimal way.

It should be emphasized that before introducing a new technology it is necessary to check whether local soil and water conservation measures already exist and why and how farmers apply these indigenous technologies. If such technologies exist and continue to be applied by farmers, then, providing they have not been introduced and maintained by legal force and state authority, they can be considered successful and on investigation will be found to provide tangible benefits. Understanding the reasons why farmers use such technologies, i.e. the production and conservation benefits they get from them, is the key to the successful introduction of any "new" technology, which must at least match and preferably improve on the benefits to be obtained from the existing ones (CARDI, 2010).

The effect of soil and water conservation measure in reducing soil loss generally varies with soil type, land use, land cover, topography, climate and intensity of the measures. Among the factor major contribution for reducing erosion is from farming system in general and land use land cover specifically. In this regard the major factors are related to every day activity of land owner/farmers/. Therefore, they protect their soil indigenously for their crop productivity. Different authors assessed many ISWC practices that can reduce soil loss however it was not organized as a form of integrating its historical analysis, source, and property, technical social, economical and cultural aspects. For this reason, this project was initiated to identify and investigate different ISWCP that could add value on reducing soil erosion and increasing moisture on farms so that, it will be documented for future development.

Therefore, the objectives of study was to identify indigenous and introduced SWC practices, to measure and describe identified indigenous and introduced soil and water conservation practices and to document the identified practices for further reference.

II. Methodology

a) Site selection

Two zones and one special district were selected for this study. Based on their agro ecological condition, farming practice and land use land type. Bonke, Boreda and Zala woredas from Gamo Gofa zone high land, midland and, lowland respectively was selected. Konso and Derashe woreda from Segen area peoples zone and Basketo special districts was also selected.

During selection of site, Focus Group Discussion (FGD) was made at zonal and woreda basis using checklist prepared for the objectives of the activity. Detail discussion was organized with zonal agricultural department so that woreda were grouped under similar farming system. Discussion was be undertaken with selected multi-disciplinary team from (NRM, Crop, Animal science, socio-economic, irrigation) who have experience about all woredas, having detail information and share on issues of farming system in the woreda. Sample of woredas having similar farming system was selected. Detail discussion was organized with woreda agricultural and natural resource office and kebelese was selected based on the detail farming system. Preliminary survey was being made using developed checklist to group kebeles in to similar farming system. Sample of kebeles with similar farming system was selected for detail study.

b) Data collection and organization

i. *Transect walk*

Transect walk was made in the selected woredas and kebele. A transect walk is a tool for describing and showing the location and distribution of resources, features, cropping and farming practices, soil and water conservation practices , landscape, main land uses along a given transect. It can be used for identifying and explaining about traditional and modern knowledge of natural resource management of the communities.

ii. *Focus group discussion (FGD)*

Focus group discussion (FGD) was made to identify the practices. A focus group discussion involves gathering people from similar backgrounds or experiences together to discuss a specific topic of interest. It is a form of qualitative research where questions are asked about their perceptions attitudes, beliefs, opinion or ideas. In focus group discussion participants are free to talk with other group members; unlike other research methods it encourages discussions with other participants.

In this study Focus Group Discussion (FGD) has taken with zone and woreda level. At zone level discussion under taken with experts from multi disciplinary teams like (natural resource, irrigation, animal science, and plant science departments) they have detail information's about all woredas of the zone so that, grouping of woredas was done based on its agro ecologies and expectation to have indigenous and introduced soil and water conservation practices. In this regard representative woredas/ districts/ were selected. Similar trend of FGD to identify sample kebeles based on the existence of indigenous and introduced soil and water conservation practices.

Figure 1: Focus group discussion in Konso (source field survey 2018)

iii. *Interview*

An interview is a conversation where questions are asked and answers are given. In common parlance, the word "interview" refers to a one-on-one conversation between an interviewer and an interviewee.

Key informant interview was made at respective administrations from zone to kebele level by participating administrators, experts and elder farmers well known to the area in order to get information on the area where soil and water conservation practices found. In other way this discussion helps to identify those farmers practicing indigenous and introduce soil and water conservation practices. The selection of those farmers was purposively based on availability of representative indigenous or introduced soil and water conservation techniques to reduce the problem of soil erosion and increase soil moisture content.

c) Data Analysis Presentation

Targeting the objective of documentation of findings, data analysis was done more with qualitative description and explanation supporting it with picture or figure. The analysis focused on discussion on zonal basis in the region so as to summarize the documentation of indigenous and introduced SWC practices and measure by using Tables and Figures.

III. Result and Discussion

a) Characterizing indigenous soil and water conservation methods in study area

i. *Physical indigenous SWC*

a. *Stone terrace*

Stone terrace are one of the physical soil and water conservation practices which are traditional well practiced in the study area. From selected woredas of study area Konso and zala has majorly practiced stone terrace for the purpose of erosion prevention. The people of Konso are a hundred of year experience of constructing stone terrace for the purpose of soil management, water harvesting, and deference wall. Most of the agricultural land and communal land are covered with different stone terrace in Konso zone.

Figure 2: "Afilmayta " in konso (source field survey 2018)

Figure 3: "korayida" in konso (source field survey 2018)

Table 1

No	Local name	Main advantages	Existing districts	Categories	Dimension				
					Av. Length (m)	Av. Depth (m)	Av. Height (m)	Av. Spacing (m)	Av. Width (m)
01	"*Kama* "	Conserving soil	Konso	Indigenous	Farm size	--------	0.3	4.5	0.2
02	"*Afilmayita* "	Fence, defence	Konso	Indigenous	1.5	1	2.5	10	1.5
03	"*korayida*"	SWC	Konso	Indigenous	Farm size	0.5	1.35	5	1.2
04	"*Shucha kela* "	SWC	Zala	Indigenous	Farm size	0.4	1	3.5	0.5

b. *Targa*" *and*" *pataya*"

The word "Targa," and " Pataya" means Derash language or Derashigna. It is an indigenous in situ moisture conservation techniques practiced by Derash people of southern Ethiopia. "Targa," and/ " pataya" means a rectangular shaped on farm moisture conservation technique in which the embankment has built from soil or plant residue (sorghum or maize straw).

In the study area the people of Dherash largely cultivated maize, sorghum and teff. Most of lowland kebles of the worda like (Kola mashile, Holite, Ateya, Nota,Walesa, Shelale, Keyama, Wolayite and Argoba) were majorly practiced "Targa," and " Pataya". The differences between "Targa," and " pataya" were only dimension.

Table 2

No	Local name	Main advantages	Existing districts	Categories	Dimension				
					Av. Length (m)	Embankment width (m)	Av. Height (m)	Av. Spacing (m)	Av. Width (m)
01	"*Targa* "	Moisture conservation	Dherasha	Indigenous	3.4	0.3	0.4	5	2
02	"*Pataya*"	Moisture conservation	Dherasha	Indigenous	1.5	0.2	0.3	2	1

Figure 3: "*Targa and Pataya* " in Dherashe *(source field survey 2018)*

c. *Cut-off drain*

A cut-off drain are earth structures constructed across a field used to intercept run off and divert surface run-*off* from the slope above and *drain* it to a safe outlet. In the study area special in Boreda woreda of Gamo zone the farmers were constructed cut- off drain on their farm land and locally called "*Dio ogiya*".


Figure 4: "*Dio ogiya* " on farm land and around home garden in Boreda *(source field survey 2019)*

Table 3

No	Local name	Main advantages	Existing districts	Categories	Dimension		
					Av. Length (m)	Av. Depth (m)	Av. Width (m)
01	" *Dio ogiya*"	Soil conservation	Boreda	Indigenous	Farm size	0.4	0.5

ii. *Agronomic indigenous SWC*

a. *Mulching*

Mulching mean leaving crop residues on the field after traditionally in the study district .The farmers in the study area traditionally leave the straw of sorghum or maize on their farm land after harvest to improve soil fertility and to conserve soil from rain drop erosion.

Figure 5: Mulching with maize & sorghum straw in Basketo sp. district *(source field survey 2019*

b. *Inter cropping*

Intercropping is a farming method that involves planting or growing more than one crop at the same time and on the same piece of land. It means having more than one type of crop growing in the same space at the same time. The most common goal of intercropping is to produce a greater yield on a given piece of land by making use of resources or ecological processes that would otherwise not be utilized by a single crop. In the study area farmers traditionally practiced sowing different crops simultaneously at the same cropping season. The major intercropping crops are maize with common bean, maize with mung bean, maize with sun flower and sorghum with other legumes in the mid and low land of study area.

Figure 6: Intercropping inter cropping maize with sun flower in Basketo sp. district *(source field survey 2019)*

c. *Crop rotation*

Crop rotation is the practice of cyclically growing a sequence of different plant species on the same parcel of land following a defined order of the crop succession with a fixed length. It is done so that the soil of farms is not used for only one set of nutrients. It helps in reducing soil erosion and increases soil fertility and crop yield.

iii. *Biological indigenous SWC*

a. *Park land agro forestry*

Agro forestry refers to a land management practice in which cultivation and use of trees and shrubs with crops and livestock in agricultural system. Agro forestry seeks positive interactions between its components, aiming to achieve a more ecologically diverse and socially productive output from the land than is possible through conventional agriculture. Farmers in the study area had good experiences on use of integrated agro forestry *Moringa Stenophetala*, *Mangifera indica, Gravelia rebusta Terminalia browenii*, *Cordia Africana*, Banana, and other fruit tree species planted traditionally based on contour line with the integration of animal fatting grasses.

Figure 7: Park land agro forestry in Zala district Gofa Zone (source field survey 2019)

b. *Home garden Agro forestry*

The home garden can be defined as a farming system which combines different physical, social and economic functions on the area of land around the family home. *Home garden* is an area of land, individually owned, surrounding a *house* and usually planted with a mixture of perennials and annuals Inset based, coffee based, root and tuber crop, fruit tree based and other types of home garden agro forestry were majorly practiced in the lowland, mid land and high land agro ecology of the study area.

Figure 8: Home garden Agro forestry in Zala district Gofa Zone (source field survey 2019)

Figure 9: Home garden Agro forestry in Bonke district of Gamo Zone (source field survey 2019

c. *Living Fence agro forestry*

Living Fence agro forestry is a technology practiced in sloping areas in which hedgerows are established along the contours and other annual/cash crops are grown in the alleys between the hedges. *Contour Hedgerows* of Nitrogen-fixing Plants and Shelter/Protection Belts to Reduce Runoff and Soil Loss. Traditional in the study area farmers had practiced planting of different nitrogen fixing trees as a fence for the purpose of soil fertility improvement and erosion protection.

Figure 10: Live fence of Korch (Erythrina abyssinica) in Basketo special district (source field survey 2019

b) *Characterizing introduced soil and water conservation methods in study area*

i. *Stone bund*

Stone bunds are used along contour lines to slow down, filter and spread out runoff water, thus increasing infiltration and reducing soil erosion. Over time sediment, which is captured on the higher side of the bunds, accumulates to form natural terraces. The farmers in the study area had practiced stone bund with advanced way mostly in stony area. Besides to that most of the stone bund structures had with biological stabilizers like elephant and desho grasses.

Figure 11: Stone bund with stabilizer desho grass in Bonke Gamo zone (source field survey 2019

Figure 12: Advance Stone bund Zala Gofa zone (source field survey 2019)

ii. *Grass strip*

Grass strip means planting different grass on contour line to slow the speed of water coming down the slope and allow the water to infiltrate. They also allow the washed away soil and nutrients to settle out above the hedgerows. In study area through the recommendation of development agents farmers locally plant desho grass on their farm lands.

Figure 13: Grass strip in Bonke Gamo zone (source field survey 2019)

iii. *Rain water harvesting pond*

The rainwater can be collected in large quantity in ferro-cement or plastic line ponds. The roof water, runoff water (after filtration) or spring water may be diverted to the pond. A large sum of water can be harvested using such ponds, which in turn may be used for irrigation or household purposes.

Figure 14: Rain water harvesting pond in Bonke Gamo zone (source field survey 2019)

iv. *Gabion*

Gabion a basket or container filled with earth, stones, or other material used for slope stability and erosion protection in construction.

Figure 15: Water harvesting pond in Bonke Gamo zone (source field survey 2019)

IV. Conclusion and Recommendation

In the study area, various indigenous and introduced soil and water conservation measures had been implementing by farmers at different land use systems. However, indigenous SWC measures taken less attention by different stakeholders; Governments, nongovernmental organization, and research institute.

Thus, based on the finding, the following suggestion may be important; farmers construct indigenous SWC without any scientific calculation, which leads to farm land fragmentation and labor intensive. Therefore, these indigenous practices need attention for further improvement.

It is better to conduct detail study on its socio-cultural values, bio-physical properties and its effectiveness on soil erosion control with reference to other practices or farmers practice and disseminate technically, economically, socially and ecologically viable indigenous knowledge of Gamo Gofa, Segen areas people's zone and Basketo sp. districts practices to the areas with a similar agro-ecology.

In similar manner, some quality and technical imperfection were also observed and evaluated on introduced SWC against standard guideline developed by MoARD. This may be due to time interval between implementation and evaluation, free grazing, lack of regular maintenance, improper design and construction, and deliberate destruction of bunds by land owners.

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14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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