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On-Station and On-Farm Screening of Bread Wheat (*Triticum aestivum* L.) Genotypes across Selected Potential Areas in Southern Ethiopia

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Abstract

Wide use of unimproved cultivars and management practices, diseases especially wheat rusts are some of the factors significantly affecting wheat production in Ethiopia. In the present study, 23 elite bread wheat genotypes and two check varieties (Danda' and Digalu) were evaluated at Hossana, Angacha, Kokate and Waka in 2014 and 2015 for grain yield and responses to stem rust. The study identified two bread wheat genotypes namely ETBW6440 with better mean grain yield ranged from 3636 to 4669 kg/ha and ETBW6188 with mean grain yield ranged from 3740 to 4917 kg/ha. ETBW6440 and ETBW6188 also exhibited 5MR and 20MR to stem rust, respectively. After seed multiplication in 2016, the two genotypes and check varieties Danda' and Wane were promoted to Variety Verification Trial (VVT) which was conducted at Hossana, Angacha and Kokate and on two farmers' fields at the vicinity of each research station using large plots in 2017. The VVT at the three stations and on farmers' fields were evaluated by national variety releasing committee, group of farmers and group of researchers. During VVT, a bread wheat genotype ETBW6188 exhibited overall better field performance and tolerable level of stem rust reaction across the three research stations and farmers' fields. Thus, ETBW6188 was preferred by all groups participated in evaluation of VVT and finally decided to release the genotype which was later given a breeder name "Bondena". Molecular analysis revealed that Bondena contained Sr31 and Sr58 which are supposed to provide all stage resistance and adult plant resistance, respectively to various stem rust races. Therefore, we recommend Bondena for production to highlands of SNNPR where the variety was evaluated including areas with similar agro-ecologies.

Keywords: Bondena; Grain yield; Stem rust resistance; Stem rust resistance genes; *Triticum aestivum*

Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most important staple cereal crops grown worldwide. In Ethiopia, wheat is the third major crop in area coverage [1]. In wheat production area, Ethiopia is the leading in sub-Saharan countries [2]. Wheat accounts for 20% of the nation's total cereal production including maize, barley, tef, sorghum and others [2,3].

Wheat production area in the country has been steadily increasing and reached to more than 1.7 million ha [4,5]. The small-scale farms contribute the major proportion against large commercial farms of wheat production where the former accounts for more than 90% of the present production area in Ethiopia [6]. Wheat productivity growth rate appeared to be too retarded and it does not exceed 2.4 tons per hectare until 2017 where it was more than 3.4 tons per hectare of world average in the same year [7]. Although the current government of Ethiopia expected to increase wheat production in the coming years, the nation still couldn't meet local demand [6]. To narrow the gap between domestic production and domestic demand, the government has been continuously importing wheat from the Black Sea region for the last several years incurring a lot of foreign hard currency [3,6].

Messay et al. reported the mean productivity of the wheat about 0.76 t/ha in SNNPR [8]. However, the productivity has been accelerated up to 2.5 t/ha in 2015/2016 implying more than 30% yield gain [4]. Although the current improvement can be seen as a notable change within 3-4 years, this productivity continued to be grumbled as still low compared to potential productivity being recorded for improved varieties in experimental plots. More than 3.50-5.00 t/ha average productivity are being reported for bread wheat from various Experimental Stations [9].

The current low productivity at national level (Ethiopia) and regional level (SNNPR) are attributed by various production constraints [3,8,10]. Messay et al. reported insufficient choice of wheat varieties in SNNPR [8]. The authors envisaged an increased choice of improved varieties appeared dependent on number and strength of Research Centres in each State of the country. However, before the release of report by Messay et al. SARI, realizing this fact, had been declared for implementation of massive adaptability tests of most of the crop varieties including wheat and other major food crops [8]. Such massive adaptation work has been conducted at several research mandate areas which are at each Research Centre administered by SARI and the approach was targeted to identify best adapted, better yielding and farmer preferred varieties. Scaling up programmes that were conducted after adaptability studies believed to contribute to the present regional average, 2.50 t/ha [11]. Recently, beside adaptability tests, variety development scheme has also laid to develop new wheat varieties for the region. Thus, the present study was initiated with the following objectives:

- To identify wheat genotypes combining better yield and diseases resistance under potential areas and select candidate varieties to promote to VVT.
- To verify and validate candidate varieties before release.

Materials and Methods

Description of the study areas

Hossana is capital city of the Hadya zone; however, the Research Station is located near the city at about 1 km at 7°34'N and 37°50'E at an altitude of 2259 meter above sea level (masl). The mean annual rainfall is 1200-1300 mm and mean annual temperature is 18°C-28°C. The dominant soil type is lixi sols.

Angacha is capital city of the Angacha district in Kemabata Tembaro zone. The Research Station is located near the town at about 0.5 km an altitude of 2400 masl. The mean annual rainfall is 1500-1700 mm and mean annual temperature is 17°C-26°C. The dominant soil type is Alfi sols.

Kokate is part of the Wolayita zone. The Research Station is at about 7 km from Soddo, capital city of Wolyta zone. The site is located at at 6°53'N and 37°48'E at an altitude of 2100 masl. The mean annual rainfall is 1200-1300 mm and mean annual temperature is 18°C-28°C.The dominant soil type is sandy loam.

Waka is part of the Dauro zone. The Research Station is located at about 9 km from Waka, capital city of Mareka district in Dauro zone. The site is located at an altitude of 2430 masl. The mean annual rainfall is 1450-1630 mm and mean annual temperature is 14 °C-25°C. The dominant soil type is sandy loam.

Crop production systems are nearly similar at each Research Station. Enset and potato are some of the major root crops grown in the vicinities of the stations. Bread wheat is one of the major cereals grown at each location.

Experimental materials

Hundreds of bread wheat genotypes developed for potential areas were procured from Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), Mexico to Ethiopia; and we received 120 genotypes from Kulumsa Agricultural Research Center to be tested under observation nursery that was conducted at Kokate in 2013. For the present study, in total, 23 bread wheat genotypes (out of the 120) were promoted to regional variety trial (RVT) based up on better grain yield and low level of infection against different wheat diseases. Including two standard checks, Danda' and Digalu, 25 wheat materials were evaluated at RVT level at four Research Stations: Hossana, Angacha, Kokate and Waka in 2014 and 2015. After two years of evaluation, two bread wheat genotypes, ETBW6440 and ETBW6188, were further promoted to VVT based up on best mean grain yield and better reaction to stem rust. The main crop season of 2016 was used to multiply seed for the two bread wheat genotypes. Bread wheat genotypes, ETBW6440 and ETBW6188, and check varieties, Danda' and Wane, were evaluated at VVT level at Hossana, Angacha and Waka Research Stations and two farmer fields in vicinities of each station in 2017.

Seed rate of 100 kg/ha was used during both experiments: RVT and VVT. A plot size of 2.5 m by 1.2 m was used for row planting spaced by 20 cm during RVT. During VVT, a high plot size, 10 m by 10 m, was used with similar planting types and row spacing. Fertilizers DAP and urea were used at rates of 100 kg/ha, respectively during RVT. However, NPS and urea were used at rates of 120 and 100 kg/ha, respectively during VVT. During both RVT and VVT, urea was applied in split (one-third during planting and two-third 35-40 days after planting). Weeds were managed by hand weeding uniformly whenever occurred.

Data collection and analysis

During RVT, a plot consisted of six rows of 2.5 m long spaced by 20 cm. Only four central rows were considered for data collection. Grain yield was recorded for the harvests obtained from the four central rows. Pgt, since it was the only prevalent disease during experiments, was recorded based up on protocol developed by Roelfs et al. [12]. Grain yield data from RVT were analysed using SAS software. Homogeneity of error variance was tested taking into account the largest error mean square and the smallest error mean square. Bread wheat genotypes ETBW6440 and ETBW6188 including check varieties Danada' and Wane were evaluated at VVT level for general field performance by various groups including NVRC, farmers and researchers. This field evaluation resulted in decision to release ETBW6188 latter named "Bondena".

DNA for newly released variety, Bondena, was extracted in molecular lab of the University of the Free State (UFS), South Africa according to protocol described by Saghai-Maroof et al. [13]. SSR markers linked to selected Sr genes were compiled and checked for their tight linkage. Those markers validated for their tight linkage were used to assay presence of selected Sr genes in a bread wheat variety Bondena and the markers were rated as present (1) or absent (0) (Table 1).

Targeted gene	Marker	Forward primer (5'.3')	Reverse primer (5'.3')	References
Sr2	csSr2	CAAGGGTTGCTAGGATTGGAAAAC	AGATAACTCTTATGATCTTACATTTTTCTG	[14]
Sr31	Iag95	CTGTGGATAGTTACTTGATCGA	CCTAGAACATGCATGGCTGTTACA	[15]
Sr42	FSD-RSA	GTT TTA TCT TTT TAT TTC'	CTCCTCCCCCCA	[16]
Sr55	Gwm 192	GGTTTTCTTTCAGATTGCGC	CGTTGTCTAATCTTGCCTTGC	[17,18]
Sr57	CSSfr5	GGGAGCATTATTTTTTTCCATCATG	ACTTTCCTGAAAATAATACAAGCA	[19]
Sr58	Barc 80	GCGAATTAGCATCTGCATCTGTTTGAG	CGGTCAACCAACTACTGCACAAC	[20]
SrTmp	Gpw5182	TCCACTTCACTAACAAACACGG	AAAAGCTGTATAGGCAGTTCGC	[16]

Table 1: Simple sequence repeat markers linked to stem rust resistance genes used in thepresent study.

Results

ANOVA using SAS software v 9.0 revealed significant differences (P<0.05) among bread wheat genotypes for mean grain yield at each Research Station, except for Kokate. Moreover, the analysis revealed significant differences among the wheat genotypes for overall mean obtained across years and locations (Table 2). All genotypes and check varieties performed well at Angacha with mean yield ranged from 3694 kg/ha for a genotype ETBW6649 to 4917 kg/ha for a genotype ETBW6188. At this Research Station, both check varieties Danda' and Digalu gave better mean grain yield of 4453 and 4623 kg/ha, respectively. High mean grain yield (across genotypes and years), 4454 kg/ha, was also obtained at Angacha followed by 3137, 2947 and 2532 kg/ha at Kokate, Hossana and Waka, respectively.

Considering overall mean grain yield for each genotype across locations and years (across eight environments), the means ranged from 2729 for ETBW6649 to 3740 for ETBW6188. For the same type of means, six genotypes namely ETBW6434, ETBW6440, ETBW6482, ETBW6544, ETBW6188 and ETBW6497 significantly out yielded all other genotypes and check varieties with their overall mean grain yield of 3584, 3636, 3547, 3625, 3740 and 3416 kg/ha, respectively. However, no significant difference was realized among those six wheat genotypes.

Assessment for reaction to wheat Pgt revealed variable disease severity (DS) ranged from 5% for ETBW6440 to 80% for Danda' and variable field responses such as MR (moderately resistant), MS (moderately susceptible) and S (susceptible) including their combined forms such as MRMS, MSS and SMS. None of the genotypes exhibited overall R (resistance). Most of the genotypes exhibited S accompanied by DS more than 20% (Table 3).

Six bread wheat genotypes, which gave highest overall mean grain yield and listed earlier also showed variability in DS and field responses. Overall DS and field responses of 30S, 5MR, 40S, 40S, 20MR and 20MS were recorded for ETBW6434, ETBW6440, ETBW6482, ETBW6544, ETBW6188 and ETBW6497, respectively (Table 3). Therefore, the study revealed that genotypes ETBW6440 and ETBW6188 with their better overall mean grain yield and disease reaction 5MR and 20MR, respectively, combined better overall mean grain yield and resistance to Pgt. These both bread wheat genotypes thus were promoted to VVT. However, at the end of VVT, the latter genotype, ETBW6188, was decided to be released by NVRC and it was given commercial name "Bondena".

DNA from newly released variety, Bondena, was extracted. All available DNA markers reported in previous studies to be linked to known Sr genes such as Sr2, Sr25, Sr31, Sr42, Sr55, Sr57, Sr58 and SrTmp were assembled and few were validated for their tight linkage (Table 1). Analysis of SSR markers linked to the Sr genes in a bread wheat variety Bondena revealed that the variety was positive for the most important ASR gene, Sr31 and APR gene, Sr58.

No	Bread wheat varieties	Average grain yield (kg/ha)				
		Angacha	Hossana	Waka	Kokate	Average grain yield combined over environments
1	ETBW6432	4073bac	3108edfc	2008f	2997	3047ef
2	ETBW6434	4902a	3179ebdc	2753ebdcf	3501	3584bac
3	ETBW6435	4672ba	2930edf	2625ebdcf	3206	3358edc
4	ETBW6440	4669ba	3661a	2945bdac	3266	3636ab
5	ETBW6481	4555ba	2658ef	2248egcf	3348	3203ed
6	ETBW6482	4860ba	3189ebdc	2948bdac	3190	3547bdac
7	ETBW6490	4460bac	2924edf	3015bac	3182	3397ebdc
8	ETBW6544	4686ba	3274bdc	3120ba	3417	3625ab
9	ETBW6120	4461bac	2717edf	2229egcf	3114	3131e
10	ETBW6166	4501ba	3034edfc	2267egcf	3143	3236edc
11	ETBW6184	4213bac	2561f	2715ebdcf	3376	3217edc
12	ETBW6185	4422bac	2638ef	2542ebdcf	3087	3173e
13	ETBW6133	4257bac	2559f	2396ebdgf	2949	3041ef
14	ETBW6188	4917a	3533bac	3374a	3125	3740a
15	ETBW6189	4371bac	2912edf	2183gf	2801	3067ef
16	ETBW6196	4289	2812edf	2371edcf	2700	3043ef
17	ETBW6337	4619ba	2996edfc	2407ebdgf	3120	3266edc

18	ETBW6339	4129bac	2619ef	2523ebdgf	3069	3085e
19	ETBW6262	4282bac	3019edfc	2641ebdcf	2949	3223edc
20	ETBW6454	4489ba	2536f	2083gf	3147	3064ef
21	ETBW6497	4567ba	3684a	2231egcf	3181	3416ebdc
22	ETBW6533	4356bac	2956edfc	2829ebdc	3496	3410ebdc
23	ETBW6649	3694	2568f	1733g	2921	2729f
24	Danda'a	4453bac	2674ef	2593ebdcf	2992	3178e
25	Digelu	4623ba	2804edf	2487ebdgf	3103	3193e
Mean		4454	2947	2532	3137	3268
CV		14.6	16.9	16.4	19.8	20.3

Table 2: Mean grain yield (kg/ha) in each and over locations in bread wheat genotypes evaluated across four locations in 2014 and 2015.

No.	Angacha		Hossana		Waka		Kokate		Overall score[a]
	2014	2015	2014	2015	2014	2015	2014	2015	
1	10S	200S	30S	TrMR	30S	10MS	10S	55S	55S
2	5S	255S	20S	TrMS	30S	10MS	5MS	5MS	30S
3	25SMS	25MS	10MS	10SMS	30MSS	5S	TrS	10SMS	30MSS
4	TrR	0	5MR	0	5MR	TrR	TrR	TrMR	5MR
5	10MS	10MS	50S	0	40SMS	5S	10MS	10MS	50S
6	10S	10S	40S	0	40S	5SMS	10SMS	TrRS	40S
7	15S	25S	20SMS	0	20SMS	45S	5SMS	10SMS	45S
8	5S	40S	20MSMR	TrS	20MSMR	40S	10MS	40SMS	40S
9	20S	20S	10SMS	TrR	10MS	55S	20MS	20SMS	55S
10	25S	45S	5MS	0	5MS	35S	50S	TrS	50S
11	5S	5S	30S	TR	5MS	25S	5MS	TrS	30S
12	20S	25S	5SMS	TrMS	5SMS	25S	10S	TrMS	25S
13	5S	15S	10SMS	30S	10SMS	10SMS	25SMS	TrS	30S
14	5MR	15MR	TrMR	0	TrMR	10MR	5RMR	20MR	20MR
15	10MS	15MS	10SMS	5SMS	15SMS	15SMS	5MR	20SMS	20SMS
16	20S	30S	25S	0	50S	15S	10S	10MS	50S
17	30S	40S	20S	5MS	20S	55S	5SMS	30S	55S
18	20S	40S	25S	15S	10SMS	60S	10SMS	25SMS	60S
19	10MS	20MS	5MS	55S	5MS	35SMS	25SMS	45S	55S
20	20S	20S	20S	TRS	20MS	20SMS	50S	45S	50S
21	TrMS	TrMS	TrMR	10MS	TrMSMR	20MS	TrMR	5MS	20MS
22	TrMS	TrMS	TrMS	10MS	25S	30SMS	TrS	40S	40S

23	TrMS	TrMS	20MRMS	10MR	20MR	10MR	TrR	10MR	20MRMS
24	5MR	35SMS	10RMR	25SMS	20MS	80S	10MR	40S	80S
25	5MR	30MS	5MRR	40MS	30MSS	60S	10MS	50S	60S
Note: Tr=trace, DS is below 5; aoverall score is the highest score (but not mean) across eight environments.									

Table 3: Stem rust score in each season and location in elite wheat genotypes evaluated at four locations in 2014 and 2015.

Discussion

Bread wheat, one of the most important staple food crops, has been used as a source of various traditional and modern food products in Ethiopia and other sub-Saharan countries [2,5]. Although majority of its production lays mainly on small scale farming in Ethiopia, the wheat has considered as a commodity crop which has been used as a source of employment and income generation for youths and women [14]. It is a crop of mainly mid to highlands of the country where the areas are characterized by fragmented wheat lands shared among many millions of households. In such cases it is essential to intensify the farming system to achieve more yields per unity area of land. The present average wheat productivity is too low, 2.50 t/ha, at national level in Ethiopia [4]. The same source reported similar average in the case of SNNPR. Low average productivity, lower than 20 t/ha, was also reported in some zones under SNNPR. Such and similar situations were used as drivers at the back for initiation of the present study to evaluate different bread wheat genotypes across various locations and years. Number of bread wheat genotypes, in the present study, exhibited higher grain yields as high as nearly two folds of the current average productivity reported in previous studies at national (Ethiopia) and regional (SNNPR) levels [4]. To improve wheat productivity, existing natural and artificial variability within a crop need to be assessed [15]. Results from the present study revealed the potential and possibility of increasing wheat productivity through varietal and seed replacement. Significance of varietal and seed replacement for yield increment at farm level was also detailed in the report released by Mathewos and Yasin [11]. However, modern variety alone cannot bring substantial yield gain with no combination of improved management practices [20-23]. Besides attempts for improvement of genetics of a cultivar, there is always a need to pinpoint other agronomic aspects where they intercept with better yield gain in a particular cultivar [23]. High yields in superior wheat genotypes identified in the current study were accompanied with application of all other improved agronomic practices such as recommended seed rate, fertilizer types and rates, weed management, etc. Although yield gain due to variety and due to improved management practices were not clearly quantified, variability observed in productivity among test lines was due to wheat genotypes with no doubt. It was because of that all genotypes were compared at similar management practices across locations and years.

From over locations and years evaluation of the wheat genotypes, six bread wheat genotypes significantly outshined all other genotypes and check varieties for grain yield. They gave relatively higher overall mean yields in a suggested improved management practice. Nonetheless, the current study did not recommend advancement of all six genotypes as they exhibited variability to disease resistance. However, only two of the genotypes (out of the six), ETBW6440 and ETBW6188, were promoted to VVT, next phase of the evaluation because these two genotypes exhibited low field infection due to Pgt. VVT is usually considered as a phase of validation where the candidate varieties including checks are often evaluated at both stations and on farms. The VVT experiments were subjected to various groups including NVRC, researchers and farmers to gather their views in order to judge whether the candidate variety qualifies to be a VARIETY for production or not. To-date, more than 100 bread and durum wheat varieties were released in Ethiopia [9]. Most of the varieties were out of production mainly due to susceptibility to ever evolving races of wheat rusts [5,24]. Diseases are the other major wheat production constraints in Ethiopia and worldwide. Most of the varieties affirmed previously for high yields have been suffered from disease outbreaks and got rid of from the production. Disease epidemics occurred in the past on major Ethiopian wheat varieties such as Dshen in 1988, Enkoy in 1993 and 1994, Galema in 2010, Digalu in 2013 and most recently Hidase in 2018 (unpublished; personal observation) has caused heavy damages on the crop [3,25-28]. The past history on effects of disease epidemics to various wheat varieties indicates importance of ongoing searches in order to develop varieties combining both disease resistance and better grain yield. Thus, timely availing of new varietal choices ahead of evolvement of new disease epidemics always appeared essential. In the present study, the newly released bread wheat variety, Bondena, was recommended because of better yield and acceptable resistance to wheat stem rust diseases across all locations and years during NVT and VVT. Better phenotypic expression against stem rust recorded at experimental field, was further confirmed by molecular analysis. All stage resistance gene Sr31 and APR gene Sr58 were detected in this variety. According to reports released by Nirmala et al. and Singh et al. some of the Sr genes including Sr31, which were defeated by race Ug99 (syn. TTKSK), were found resistant to the new non-Ug99 race TKTTF, one of the aggressive Pgt races that was discovered in Ethiopia in 2013 [27-29]. Sr58, an APR gene, was also reported to be efficient to provide resistance against Ug99 and non-Ug99 races [27]. Therefore, the newly released variety, Bondena, can be one of good choices not only for further production, but it can also be used as a source for important disease resistance genes for further gene pyramiding programs.

Conclusions and Recommendations

Currently, wheat yield reduction got special concern by Ethiopian government. Population growth and tendency of Ethiopian people to change lifestyle at both rural and urban population has led to high demands resulted in obligation to import more wheat grain from abroad through incurring a lot of foreign currency which otherwise would be used for other developmental purposes. To narrow or close difference between local demand and local supply, appropriate strategies need to be put in a place. System intensification in cases of small-scale farmers and exploitation of unused land resource in lowlands through laying irrigation schemes has hopped to raise local production to the level of current demand. To raise wheat productivity in already existing lands, development of better yielding varieties combining resistance to diseases is the priority agenda in wheat improvement research in developing and developed countries. The present study delivered a new bread wheat variety, Bondena which gave better yield across eight environments and finally judged during VVT to be released by various groups composed of NVRC, farmers and researchers. Apart from due to other uncontrolled disasters such as outbreak of diseases, droughts and/or other calamities, it must be cautioned that the current productivity in any new varieties can be affected negatively for any deviation in application of improved management practices. Therefore, we recommend bread wheat variety Bondena with improved management practices for wider production in highlands of SNNPR.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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