World Open Research Journal of Soil Science
Vol. 1, No. 1, March 2017, pp. 1-9
Available online at http://scitecpub.com/Journals.php

Research article

The significance of inoculation with a mixture of mycorrhiza, phosphorous solubilizing bacteria and Trichodermaunder application of different phosphorous levels on growth and yield of maize (*Zea mays* L.)

M.A.M. Adlan[1], K.A. Eltaib[2], H.K. Ali[3], O. Elsiekh[4], A.M. Magboul[1], Ahlam.E. Ahmed[1] and M.A. Salih[1]

[1]Gezira Research Station, [2]Dongola Research Station, [3]Hudieba Research Station, [4]New Hamdab Research Station

E-mail: aelsammani2003@yahoo.com

This work is licensed under a Creative Commons Attribution 4.0 International License.

Abstract

A field experiment was conducted during the winter season of 2015 at the Research Farm of Dongola, New Hamdab and Hudieba High Terrace soil of ARC Sudan to examine the effect of the PANORAMIX^{maize}biofertilizer on growth and yield of maize under different levels of phosphorous. The experimental design was an RCB, and the treatments were factorial combinations of three phosphorous levels (0P, ½P and 1P), applied as DAP, and two PANORAMIX^{maiz} rates (0 and 2mls/kg seeds) in four replicates. The results indicated significant interaction effect of P and biofertilizer PANORAMIX on grain yield of maize. The combined application of the PANORAMIX with 1P significantly improved the grain yield by 119, 127 and 123% over the control at Dongola, Hamdab and Hudieba, respectively, and it significantly increased the grain yield by 44, 24 and 10% compared to 1P alone at Dongola, Hamdab and Hudieba, respectively. Furthermore, inoculation of maize seeds with PANORAMIX in the absence of P fertilization resulted in grain yield higher than that obtained at 1P at Dongola, compared to that of 1P at Hudieba, and significantly higher than that of 1/2P at Hamdab. These findings may nominate the

Copyright ©scitecpub.com, all rights reserved.

PANORAMIX^{miaze}biofertilizer as a good candidate for sustainable production of maize and as a friendly environment fertilizer.

Keywords: Mycorrhiza, P solublizing bacteria, Trichoderma, Phosphorous and PANORAMIX Maize

Introduction

Maize (*Zea mays* L.) is one of the most important cereals in the world. It ranks fourth after sorghum, wheat and millet in Sudan. The crop is used for food, oil, starch, ethanol and as feed for livestock. The area grown to the crop in Sudan is estimated at around 80000 ha, with an average production of 0.75 t/ha (FAO, 2003).

Phosphorous is one of the essential elements for plant growth and development. It is involved in many important biochemical processes such as energy metabolism, photosynthesis, respiration, nutrient movement within the plant and transfer of genetic characteristics. Unfortunately, the soils of Sudan are known to be low in available P. Thus, the crops are likely to respond positively to the application of mineral P fertilizers. Following this hypothesis, maize response to P- mineral fertilizers was documented by some researchers (Salih*et al.*, 2009 and Abbas *et al.*, 2007). The drawback of regular application of mineral P fertilizers, to raise the low soil available P and therefore the yield of crops, is costly and hazardous to the environment (Pizzeghello*et al*., 2011). Not only that, but also the easily accessible high grade phosphate rock reserves are likely to be depleted within the end of this century (Schröder*et al.*, 2011). The progressively proposed alternative technology that could sustain the environment, eliminate or reduce the mineral P fertilizer need and improve the crop yield to the use of mineral P fertilizers is the biofertilizer (Covacevich 2007 and Vincenza*et al.*, 2013).

Biofertilizers play an important role on plant nutrition through transformation, mobilization and solubilization of soil nutrients. For instance, mycorrhiza mobilize P from far distances beyond the reach of plant roots to the plant, phosphorous solubilizing microorganisms secrete acids to dissolve the unavailable precipitated P to plant and Trichoderma solubilizes the phosphate, micronutrients and minerals such as Fe, Mn and Mg that enhanced nutrient uptake (Hoyos-Carajal*et al.*, 2009). In addition, these microbes improved the tolerance of the crops to biotic and abiotic stresses (Mastouri*et al*., 2010). Mycorrhiza was found to enhance, significantly, the yield of common bean (Adlan, 2008), faba bean (Ahmed *et al*., 1998) and growth of soy bean (Mahdi et al 2004). Similarly, phosphorous solubilizing microorganisms (PSM) significantly improved the growth and yield of groundnut (Mohamed and Abdalla 2013). The objective of this study is to investigate the effect of inoculation of maize with a mixture of biofertilizers (different spp. of mycorrhiza, P solubilizing bacteria and Trichoderma) on growth and yield of maize.

Materials and methods

A field experiment was carried out during the winter season, 2015/2016, at the Research Farms of Dongola, New Hamdab and Hudieba high terrace soil, of the Agricultural Research Corporation, Sudan. The design used was Randomized Complete Block and the treatments were factorial combinations of DAP and PANORAMIX^{maize}fertilizers in four replications. Factor A was three levels of P fertilizers, 0P, ½P and 1P as DAP (diammonium phosphate) and factor B was the PANORAMIX^{maize}biofertilzer at two rates (with or without). The PANORAMIX^{maize}biofertilizer contains a mixture of beneficial microbes. These are mycorrhiza (a mixure of Glomusspp: *mosseae*, *fasciculatum*, *intraradices*, *aggregatum* and *etunicatum*), Trichodermasp (*harzianum* T22)

Copyright ©scitecpub.com, all rights reserved.

and Bacillus spp (*subtilis* and *megaterium*). The size of the inoculums was more than 1000 propagules, $2x10^8$cfuper ml and $4x10^8$cfu per ml for mycorrhiza, Trichodermaspp and Bacillus spp, respectively. The product was manufactured byKoppert B.V, Veilingweg 14, NL 2650 AD Berkel&Rodenrijs, The Netherland and provided through its agency Khalil Osman Agricultural and Animal Processing Co. Ltd, Khartoum, Sudan.Phosphorous was banded with the seeds at sowing. Inoculation was made by addition of the biofertilizer liquid to the seeds at the rate of 2ml/ kg seeds, thoroughly mixed, left to dry under shade and immediately sown and irrigated. Nitrogen at the rate of 2N in form of urea after subtraction of the amount of N included in the respective P treatment was applied in two equal doses 7 days after sowing (DAS) and one month later. Seeds of maize variety Hudieba 2 were sown on the top of 60 cm wide ridges and 20 cm intra row spacing. Two seeds were sown per hole and thinned to one seedling per hill two weeks after sowing. Data collected included composite soil sample (0-30 cm) prior to sowing, plant growth parameters, yield components, straw and grain yield. The data were subjected to Analysis of Variance using Gen Stat 12th edition soft

Results and discussion

The soil properties at the three locations indicated that these soils are non saline, non sodic, alkaline, and have low organic carbon, total soil nitrogen and low available P (Table 1). The low available soil P increases the probability for significant response of maize to P mineral fertilizer and biofertilizers application. Indeed, inoculation of the maize seeds resulted in significant increase in plant height at the three locations (Table 2). The 1000-seed weight was either significantly affected by levels of P atDongola, P and PANORAMIX at Hamdab or not affected at all at Hudieba (Table 3).The use of the biofertilizers resulted in significant interaction effect between mineral P levels and biofertilizer inoculation on grain yield at Dongola, Hamdab and Hudieba, and straw yield at Hudieba (Tables 4 and 5). The combined treatment of 1P and inoculation with biofertilizer was the best one among the others that significantly improved the grain yield at Dongola, Hamdab and Hudieba, and straw yield at Hudieba. Highly significant responses for grain yield at Dongola and straw yield at Hamdab and Hudieba were obtained due to the use of biofertilizer (Tables 4 and 5). It could be stated that the combined application of the PANORAMIX with 1P significantly improved the grain yield by 119, 127 and 123% over the control at Dongola, Hamdab and Hudieba, respectively, and it significantly enhanced the grain yield by 44, 24 and 10% compared to 1P alone at Dongola, Hamdab and Hudieba, respectively.

The use of commercial mycorrhiza as a biofertilizer increased the soil available P concentration in absence and presence of P application and hence improved maize growth and yield (Cozzolino*et al*., 2013). Our findings were in line with these results where the use of commercial biofertilizer containing mycorrhiza significantly enhanced growth and yield of maize. Koide and Mosse (2004) showed that inoculation with mycorrhiza is important when the native mycorrhiza potential in the soil is inadequate in quantity and quality. Furthermore, the added commercial mycorrhiza inoculums might interact with native soil mycorrhiza and alter their function (Cozzolino*et al*., 2013). In the soil of Sudan, increasing the quantity of the inoculums through isolation, culturing and inoculation from native soil mycorrhiza resulted in significant crop response (Adlan 2008, Ahmed *et al*., 1998). This might indicate the low quantity of the native soil mycorrhiza. Also, the method used for culturing and inoculation was tedious and require bulk quantities of inoculums. The PANORAMIX seed inoculation is easy and required a few mls of inoculums size, 2ml/kg seeds.

The use of PSM significantly increased plant height by 19%, grain yield by 44%, biomass by32% and P content by 56% (Viruel et al 2014). This might be due to the ability of these microbes to increase the availability of plant nutrients such as P through acidification, chelation, exchange reaction and production of siderphore and phytohormones (Viruel et al 2011 and Rodriguez and Fraga 1999). This could explain the role of phosphorous solubilizing bacteria included in the applied constridum of PANORAMIX biofertilizer. The increase in grain yield

Copyright ©scitecpub.com, all rights reserved.

and biomass due to the application of biofertilizer alone over the control was 53 -104% and 31-65%, respectively, at the three sites of the present study (Tables 4 and 5).

It is interesting to observe that inoculation of maize seeds with the PANORAMIX biofertilizers in the absence of mineral P produced grain and straw yield similar to or greater than that of 1P alone at Dongla and Hudieba (Tables 4 and 5). This treatment, inoculation of maize seeds in absence of mineral P, also resulted in significantly higher grain yield than that of ½P and gave straw yield not significantly different from that of 1P at Hamdab (Tables 4 and 5). In line with this finding, Cozzolino*et al*., (2013) documented that inoculation with mycorrhiza in the absence of P application resulted in plant growth, grain yield and P uptake similar to those of P treatment. Furthermore, P solubilizing bacteria, P. *tolaasii*, was found to be more efficient as inoculant without P fertilizer than with TSP (Viruel*et al*., 2014). This might be due to the fact that application of P reduced the potential ability of inoculants as biofertilizer (Virueletal 2014 and Krey*et al*., 2013). Improvement of growth and yield of maize in response to inoculation with biofertilizer could, however, be obtained in absence and presence of P application (Cozzolino*et al*., 2013).

Economic evaluation

Partial,dominance andmarginalanalysis were conducted for grain yield (kg/ha) data of maize for Dongola, Hamadab and Hudieba sites. Results showed that treatment of PANORAMIX inoculums and 1P addition significantly resulted in the highest grain yield of maize in the three sites, and higher net benefit at both sites of Hamadab and Hudieba(Tables 6-9).Returntoinvestmentinthese treatments wasestimatedintheformofmarginal rate of return(MRR),which cameouttobe 20.13 for the grain yield of maize in Dongola for thetreatment o f 0P and PANORAMIX inoculums, 30 in Hamdabfor thetreatmento f ½ Pand PANORAMIX inoculums and 9.8 in Hudieba for thetreatmento f ½ Pand without PANORAMIX inoculums. The absence of 1P from treatments recorded the highest MRR explained by the additional costs of fertilizer. These results indicated the profitability andsuperiority of application of PANORAMIX biofertilizer to maize seeds. Application of PANORAMIX biofertilizer to maize seeds is, therefore, recommendedasthemoststableandeconomically feasiblefor maizeproduction inDongola, Hamadab and Hudeiba areas.

Conclusions

- The use of PANORAMIX inoculum significantly increased the plant height at the three locations.
- The combined application of the PANORAMIX with 1P significantly improved the grain yield by 119, 127 and 123% over the control at Dongola, Hamdab and Hudieba, respectively, and it significantly enhanced the grain yield by 44, 24 and 10% compared to 1P alone at Dongola, Hamdab and Hudieba, respectively.
- Inoculation of maize seeds with PANORAMIX in the absence of P fertilization resulted in grain yield greater than that by 1P at Dongola, almost similar to 1P at Hudieba and significantly higher than 1/2P at Hamdab.
- Application of PANORAMIX maize biofertilizer to maize seeds recommendedasthemoststableandeconomically feasiblefor maizeproduction inDongola, Hamdab and Hudieba areas.

Recommendations

The finding of this study suggests the following recommendations:

- Application of PANORAMIX^{maize}biofertilizer to maize seeds at the rate of 2ml/kg seeds with addition of 1P in the form of DAP for yield maximization.

Copyright ©scitecpub.com, all rights reserved.

- The use of PANORAMIX^{maize}biofertilizer without P fertilization for getting reasonable and higher yield than the control and eliminating the addition of mineral P fertilizer.

References

Abbas, A.E., Hamad, M.E., Babiker, H.M. and Nour, A.E. 2007.Effect of added phousphorous and Zinc on yield and its components of corn. Gezira J. of Agric. Sci. 5(2)126-136.

Adlan, M.A.M. 2008.Effect of biological, organic and mineral fertilizers on nodulation, N2-fixation, growth and yield of common bean (*Phaseolus vulgaris* L.). Ph.D. Thesis. University of Gezira, Sudan.

Ahmed, A.E., Elsheik, E.A.E. 1998. Effect of biological and chemical fertilizers on growth and symbiotic properties of faba bean (*Viciafaba*) under salt stress.University of Khartoum Journal of Agricultural Science.6(1) 150-106.

Covacevich, F., Echeverria, H.E., Aguirrezabal, L.A.N. (2007). Soil available phousphorousstatus determine indigenous mycorrhizal colonization of field and glasshouse grown spring wheat from Argentina. Applied Soil Ecology 35, 1-9.

Cozzolino, V., Di Meo, V., Piccolo, A. (2013).Impact of arbuscularmycorrhizal fungi applications on maize production and soil phosphorous availability.Journal of Geochemical Exploration. 129, 40-44.

Hoyos-Carvajal, L., Ordua, S., Bissett, J. (2009).Growth stimulation in bean (*Phaseolus vulgaris* L.) by *Trichoderma*Biol. Control 51, 409-416.

Koide, R.T., Mosse, B. (2004). A history of research on arbuscularmycorrhizal symbiosis. New Phytologist 147, 145-163.

FAO (2003).Food and Agricultural Organization.Statistical Year Book, 2003. Rome, Italy.

Krey, T., Vassileo, N., Baum, C., Eichler-Löbermann, B. (2013).Effect of long-term phosphorous application and plant growth promoting rhizobacteria on maize phoushporous nutrition under field condition.Euro. J. Soil Biol. 55, 1124-1130.

Mahdi,A.A., NurEldaim, A.B. and Arbab, A.A. 2004. Influnce of mycorrhizal inoculation, form of phosphate fertilization and watering regime on nodulation and yield of soybean. U. of K. J. Agric. Sci. 12(3)345-356.

Mastouri, F., Bjorkman, T., Harman, G.E. (2010). Seed treatments with *Trichodermaharzianum* alleviate biotic, abiotic and physiological stresses in germinating seeds and seedlings. Phytopathology , 1001213-1221.

Mohamed, S.S. and Abdalla, A.S. 2013. Growth and yield response of groundnut (*Arachishypogaea* L.) to microbial and phosphorous fertilizers. Journal of Agri-Food and Applied Sciences, 1(3)78-85.

Pizzeghello, D., Berti, A., Nardi, S., Morari, F., (2011).Phosphorous forms and P-sorption properties in three alkaline soils after long-term mineral and manure applications in north-eastern Italy.Agriculture, Ecosystems and Environment 141, 58- 66.

Rodriguez, H., Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion.Biotechnol. Adv. 17, 319-339.

Copyright ©scitecpub.com, all rights reserved.

Salih, M.A., Ali, E.S., Babiker, E.A., ALI, N.A. 2009. Response of two maize (*Zea mays* L.) varieties to varying rates of two phousphorous sources in Sudan, Gezira. Sudan Journal of Agricultural Research. 13, 23-36.

Schröder, I.I., Smit, A.I., Cordell, D., Rosemarin, A., (2011). Improved phosphorous use efficiency in agriculture: a key requirement for its sustainable use.. Chemosphere 84, 822-831.

Viruel, E., Lucca, M.E., Siňeriz, F. (2011). Plant growth promotion traits of phosphobacteria isolated from Puna, Argentina. Arch. Microbiol. 193, 489-496.

Viruel, E., Erazzū, L.E., Martinez Calsina, L., Ferrero, M.A., Lucca, M.E., Siňeriz, F. (2014).Inoculation of maize with phosphate solubilizing bacteria effect on plant growth and yield. Journal of Soil Science and Plant Nutrition, 14(4), 819-831

Copyright ©scitecpub.com, all rights reserved.

World Open Research Journal of Soil Science
Vol. 1, No. 1, March 2017, pp. 1-9
Available online at http://scitecpub.com/Journals.php

Table 1. Some soil properties of the selected locations

Location	Clay (%)	Silt (%)	Sand (%)	pH	EC dS/m	$CaCO_3$	ESP	O.C (%)	% soil N	Available P (ppm)
Dongola	33	20	47	7.6	0.4	8.0	3.0	0.16	0.047	2.0
Hamdab	30	5	65	8.2	0.81	4.0	2.4	0.078	0.02	4.2
Hudieba	45	3	52	8.4	0.09	8.0	3.2	0.181	0.021	3.2

Table 2. Effect of PANORAMIX inoculum and phosphorous application on plant height(cm) of maize

Site	Dongola			Hamdab			Hudeiba		
Treat	(-) PAN	(+) PAN	**Mean**	(-) PAN	(+) PAN	**Mean**	(-) PAN	(+) PAN	**Mean**
0P	141.6	152.9	**147.2**	120.3	131.1	**125.7**	134.8	178.3	**156.5**
½ P	156.7	156.0	**156.3**	136.7	130.4	**133.5**	147.5	165.8	**156.6**
1P	124.3	145.8	**135.0**	117.9	135.4	**126.6**	161.4	180.5	**170.9**
SE±	5.26ns		3.72**	3.63**		2.57ns	8.86ns		6.26ns
Mean	**140.9**	**151.7**		**125.0**	**132.3**		**147.9**	**174.9**	
	3.04*			2.10*			5.11**		
C.V. (%)	7.2			5.6			11.0		

*, ** and *** indicates statistical significance at 1, 5 and 0.1% probability level, respectively. Ns is not statistically significant at 5% probability level. PAN stands for PANORAMIX, - for without and + for with.

Table 3. Effect of PANORAMIX inoculums and phosphorous application on 1000 seed weight (g) of maize

Site	Dongola			Hamdab			Hudeiba		
Treat	(-) PAN	(+) PAN	**Mean**	(-) PAN	(+) PAN	**Mean**	(-) PAN	(+) PAN	**Mean**
0P	233.5	236.6	**235.0**	220.0	243.8	**231.9**	144.9	157.9	**151.4**
½ P	263.6	248.8	**256.2**	250.0	247.5	**248.7**	153.1	151.4	**152.2**
1P	251.6	243.8	**247.7**	236.2	280.0	**258.1**	159.2	166.7	**162.9**
SE±	6.51ns		4.61*	4.52ns		3.20***	6.33ns		4.47ns
Mean	**249.6**	**243.1**		**235.4**	**290.4**		**152.4**	**158.7**	
SE±	3.76ns			2.61***			3.65ns		
C.V. (%)	5.3			3.7			8.1		

*, ** and *** indicates statistical significance at 5, 1 and 0.1% probability level, respectively. Ns is not statistically significant at 5% probability level. PAN stands for PANORAMIX, - for without and + for with.

Table 4. Effect of PANORAMIX inoculums and phosphorous levels on grain yield (kg/ha) of maize

Site	Dongola			Hamdab			Hudeiba		
Treat	(-) PAN	(+) PAN	**Mean**	(-) PAN	(+) PAN	**Mean**	(-) PAN	(+) PAN	**Mean**
0P	1050	2147	**1599**	2329	3517	**2923**	1032	1935	**1484**
½ P	1604	2278	**1941**	3065	5070	**4068**	1737	2243	**1991**
1P	1597	2304	**1951**	4274	5284	**4779**	2099	2309	**2204**
SE±	(72.3)*		(51.1)**	(136.4)**		(96.4)***	(73.77)***		(52.16)***
Mean	**1380**	**2324**		**3223**	**4856**		**1623**	**2163**	
SE±	(41.8)**			(78.7)***			(42.59)***		

Copyright ©scitecpub.com, all rights reserved.

World Open Research Journal of Soil Science
Vol. 1, No. 1, March 2017, pp. 1-9
Available online at http://scitecpub.com/Journals.php

C.V. (%)	8.0	7.0	7.8

** and *** indicates statisticalsignificance at 1 and 0.1% probability level, respectively. Ns is not statistically significant at 5% probability level. PAN stand for PNAROMIX, - for without and + for with.

Table5. Effect of PANORAMIX inoculums and phosphorous levels on straw yield (kg/ha) of maize

Site	Dongola			Hamdab			Hudeiba		
Treat	(-) PAN	(+) PAN	**Mean**	(-) PAN	(+) PAN	**Mean**	(-) PAN	(+) PAN	**Mean**
0P	3429	4479	**3954**	2550	3853	**3201**	2188	3611	**2899**
½ P	3710	5088	**4399**	3324	4704	**4014**	3264	3958	**3611**
1P	3771	5308	**4539**	4271	6252	**5262**	3715	4028	**3872**
SE±	$(299.2)^{ns}$		$(211.6)^{ns}$	$(158.1)^{ns}$		(111.8)***	(173.2)*		(122.5)***
Mean	**3637**	**4958**		**3382**	**4936**		**3056**	**3866**	
SE±	(172.7)***			(91.3)***			(100)***		
C.V. (%)	13.9			7.6			10		

*** indicates statistical significance at 1 and 0.1% probability level, respectively. Ns is not statistically significant at 5% probability level. PAN stand for PANORAMIX, - for without and + for with.

Table 6. Partial and dominance for grain yield of maize for Dongola site

Treatment	Yield (kg/ha)	Gross return (SDG)	Variable cost (SDG)	Net return (SDG)
0P0PAN	1050	5,513	-	5,513
½P0PAN	1604	8,421	341	8,080
0P1PAN	2147	11,272	476	10,796
1P0PAN	1597	8,384	683	7,702
½P1PAN	2278	11,960	817	11,142
1P1PAN	2304	12,096	1,159	10,937 D

1 Kg equals 5 SDG
1 sack of DAP (50 kg) equals 365 SDG
1sack of urea (50 kg) equals 300 SDG
Dose of PANORAMIX inoculums equals 476 SDG

Table 7. Marginal analysis for grain yield of maize for Dongola site

Treatment	Yield (kg/ha)	Gross return (SDG)	Variable cost (SDG)	Net return (SDG)	MB	MC	MRR
0P0PAN	1050	5,513	-	5,513	-		
½P0PAN	1604	8,421	341	8,080	2,567	341	7.52
0P1PAN	2147	11,272	476	10,796	2,716	135	20.13
½P1PAN	2278	11,960	817	11,142	346	341	1.02

Copyright ©scitecpub.com, all rights reserved.

World Open Research Journal of Soil Science
Vol. 1, No. 1, March 2017, pp. 1-9
Available online at http://scitecpub.com/Journals.php

Table 8. Marginal analysis for grain yield of maize for Hamadab site

Factor	Yield (kg/ha)	Gross return (SDG)	Variable cost (SDG)	Net return (SDG)	MB	MC	MRR
0P 0PAN	2329	12,227	-	12,227	-		
½P 0PAN	3065	16,091	341	15,750	3,523	341	10.3
0P 1PAN	3517	18,464	476	17,988	2,238	135	16.6
1P 0PAN	4274	22,439	683	21,756	3,768	206	18.3
½ P 1PAN	5070	26,618	817	25,800	4,044	135	30.0
1P 1PAN	5284	27,741	1,159	26,582	782	341	2.3

Table 9. Marginal analysis for accumulated for grain yield of maize for Hudeiba site

factor	Yield (kg/ha)	Gross return (SDG)	Variable cost (SDG)	Net return (SDG)	MB	MC	MRR
0P 0PAN	1032	5,418	-	5,418			
½ P 0PAN	1737	9,119	341	8,778	3,360	341	9.8
0P 1PAN	1935	10,159	476	9,683	905	135	6.7
1P 0PAN	2099	11,020	683	10,337	655	206	3.2
½P 1PAN	2243	11,776	817	10,958	621	135	4.6
1P 1PAN)	2309	12,122	1,159	10,964	5	341	0.0

Copyright ©scitecpub.com, all rights reserved.