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Full Length Research Paper

Genotype-environment interaction and stability analysis in Wheat (*Triticum aestivum* L.) for protein and gluten contents

N. Saleem, M. Ahmad*, S. A. Wani, R. Vashnavi and Z. A. Dar

Division of Genetics and Pant Breeding, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Shalimar Campus, Srinagar -191 121, India.

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Genotype-environment interaction and stability performance were investigated on protein and gluten contents in three environments. Genotypes showed important differences in quality values as reflected in the AMMI (additive main effects and multiplicative interaction) analysis biplot result. The protein content showed the similar trend of GE interaction as that of grain yield. The genotype G1 was tightly grouped with E3 as indicated by their origin on the biplot. All the genotypes except G1 were located in the point farthest from the center of the biplot (PC1), indicating high gluten content, but the length of its PC2 vector exhibits this variety's instability, while G1 was in the center of the biplot exhibiting high stability but lower gluten level than the above mentioned cultivars. However, all the genotypes were tightly grouped with E2 with regard to gluten content and as such highly stable to this particular environment. Protein and gluten content were significantly affected by the wheat varieties under various locations. The highest protein content (pooled) was exhibited by SKW-489 (13.54%) and SW-1 (13.23%) whereas the lowest protein content was observed in SKW-848 (10.31%). Similarly, highest gluten content (pooled) was observed in SKW-517 (29.65%) and SW-355 (29.14%), while lowest percentage was exhibited by SKW-489 (22.22%).

Key words: Protein content, gluten content, stability analysis, multiplicative interaction, wheat.

INTRODUCTION

Most of the currently cultivated wheat varieties belong to hexaploid wheat (*Triticum aestivum* L.), which is known as common bread wheat and valued for bread making. The greatest portion of the wheat flour produced is used for bread making. Wheat grown in dry climates is generally hard type, having protein content of 11 to 15% and strong gluten (elastic protein). The sticky gluten of bread wheat entraps the carbon dioxide (CO₂) formed

during yeast fermentation and enables leavened dough to rise. The hard type of wheat produces flour best suited for bread making. The wheat of humid areas is softer, with protein content of about 8 to 10% and weak gluten. The softer type produces flour suitable for cakes, crackers, cookies, pastries and household flours. Durum wheat (*T. turgidum* L.), which is the main tetraploid type, is also important, although its large, very hard grains yield

*Corresponding author. E-mail: drmushatqskuastk@rediffmail.com

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low gluten flour that is the main source of semolina suitable for pasta, couscous, burghul and other Mediterranean local end-products (Nachit, 1992). Apparently, no economically important diploid wheat is being cultivated as a crop anywhere in the world. Although most of the wheat is grown for human food, however, 10% is retained for seed and industry (for production of starch, paste, malt, dextrose, gluten). Wheat grain contains all essential nutrients; kernel contains about 12 percent water, carbohydrates (60 to 80% mainly as starch), proteins (8 to 15%) containing adequate amounts of all essential amino acids (except lysine, tryptophan and methionine), fats (1.5 to 2%), minerals (1.5 to 2%), vitamins (such as B complex, vitamin E) and 2.2% crude fibers (Anjum and Walker, 2000).

Stability performance of genotypes will be of special importance in Jammu and Kashmir where environmental conditions vary considerably and the means of modifying the environment are inadequate. The major problem of bean improvement program in this state has been the lack of genotypes that consistently perform well across different bean growing environments. Hence, the development of superior quality genotypes and information on multi location performance are of paramount importance in Jammu and Kashmir where environments vary greatly within short distances. The adaptability of a variety over diverse environments is usually tested by the degree of its interaction with different environments under which it is planted. A genotype is considered more adoptive when it has a high mean yield and low fluctuations when grown over diverse environments (Ahmad et al., 2014a).

The AMMI model (Gauch and Zobel, 1997) is more efficient in determining the most stable and desirable quality and high yielding genotypes in multi-environment trials compared to earlier procedures (Eberhart and Russel, 1966). Biplot analysis is possibly the most powerful interpretive tool for AMMI models. Biplots are graphs where aspects of both genotypes and environments are plotted on the same axes so that interrelationships can be visualized. The AMMI biplot where the main effects (genotype mean and environment mean) in X axis and IPCA1 scores for both genotypes and environments are plotted in Y axis. The effectiveness of AMMI procedure has been clearly demonstrated (Crossa et al., 1990 and Tarakanovas and Ruzgas 2006).

The main objectives of the present investigation are to identify desirable quality genotype and to determine the areas where these genotypes would be adapted and economically sustainable.

MATERIALS AND METHODS

The present investigation was carried out during *Rabi* season of 2012-13 at three locations. The basic material for the present investigation comprised of 10 genotypes of wheat (*Triticum aestivum* L.) are designated as G1 (SKW-848), G2 (SKW-489), G3

(SKW-490), G4 (SKW-514), G5 (SKW-515), G6 (SKW-517), G7 (SKW-519), G8 (SKW-527), G9 (SKW-530), and G10 (SKW-531) and environments as E1 (Experimental Farm of the Division of Plant Breeding and Genetics, SKUAST-K, Shalimar, Srinagar), E2 (Mountain Field Crop Research Centre, Khudwani) and E3 (Regional Research Station, Wadura, Sopore). The experiment was laid out in a completely randomized block design with 3 replications at each location. The experimental plot comprised 3 rows each of 1 m length. Row to row and plant to plant spacing was maintained at 25 cm and 10 cm, respectively. Recommended agronomic practices were followed to raise a good crop at all the three locations. Based on the performance of the cultivars three random environments, phenotypic stability was worked out by following models (i) the AMMI model of Gauch and Zobel (1988), and (ii) the linear model of Eberhart and Russel (1966).

Protein content (%)

The grains were dried in oven and ground to the fine powder to pass through, 40 mesh sieve in a 'Micro Willey Mill.' From each treatment, 0.5 g sample was weighed for chemical analysis to determine the contents of nitrogen. The total content of nitrogen was estimated by Kjeldahl method as outlined by Campbell (1986) and was expressed in %. Protein content (%) in grain was determined by multiplying the nitrogen % in grain with the conversion factor 6.25.

Gluten content (%)

Gluten in sample of flour was estimated by washing the dough free of starch, sugars, water soluble proteins and other minor components. The wet cohesive mass obtained is referred to as wet gluten while the dry product obtained from it is referred to as dry gluten. 25 g flour was kneaded with about 15 ml of water to get a dough ball. The dough ball was allowed to remain immersed in water for one hour to ensure proper hydration after which the starch is washed out by kneading gently in a gentle steam of water over a fine sieve or silk till the washed liquid is clear.

The gluten which is cohesive was pressed as dry as possible and weighed. The gluten so obtained was dried at 100°C for 24 h and weighed again to get the value for dry gluten.

$$\text{Wet gluten (\%)} = A / C \times 100$$

$$\text{Dry gluten (\%)} = B / C \times 100$$

Where,

A = Weight of wet gluten, B = Weight of dry gluten, C = Weight of flour.

RESULT AND DISCUSSION

The results of AMMI analysis for protein and gluten content traits in wheat (*T. aestivum* L.) for the 10 genotypes and 3 environments are presented in Table 1. The AMMI analysis of data revealed that the environment, genotype, and GE interaction are highly significant ($P < 0.01$). The large MS of environments indicated that the environments are diverse. The large differences among environmental means caused in studied traits. In the present investigation, the AMMI analysis showed that protein and gluten content traits are highly influenced by genotype, environment and GE

Table 1. AMMI analysis of variance for protein content and gluten content in wheat (*Triticum aestivum* L.).

Source of variation	Df	Mean sum of squares	
		Protein content (%)	Gluten content (%)
Genotypes	9	3.46**	15.00**
Environments	2	2.69**	5.43**
Replications within environments	6	0.49*	4.38*
Genotype x Environment	18	0.08*	3.36*
Error	54	1.51	9.37
Total	89	1.38	8.29

*,** Significant at 5 and 1 per cent levels, respectively.

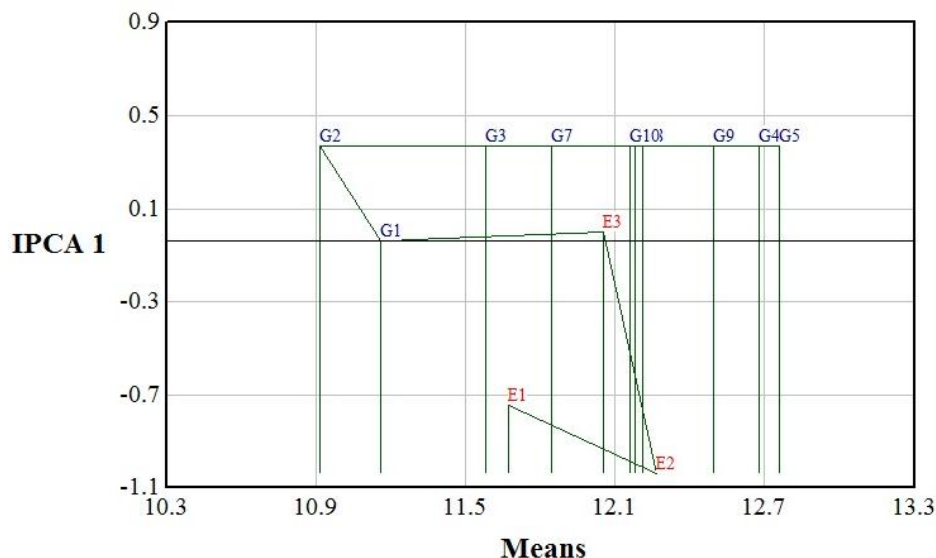


Figure 1. Biplot of the first AMMI interaction (IPCA 1) score (Y-axis) plotted against mean protein content (%) (X-axis) for 10 wheat genotypes.

interaction. Environment had the largest contribution to the total sum of squares indicating that the environments = (location and season) selected for this study are highly diverse, and this were consequently the main effect contributing most variation for these traits. The environment differences in terms of key climate attributes (temperatures and rainfall distribution), altitude and soil fertility affected the performance of wheat genotypes, justifying the need to identify high quality genotypes that are stable in a wide range of environments, or to breed for specific adaptation to specific environments. The magnitude of variation due to environments on the traits is large thereby causing genotypic response to diverse environments and suggesting the presence of mega-environments where best performing genotypes could be selected more efficiently. To characterize GE interaction, an AMMI 1 biplot are plotted using the genotype and environment mean protein content and their IPCA 1 scores (Figure 1). All the genotypes (with IPCA 1 "+") except G1 exhibited highest contribution to GE interaction

as indicated by their distance from the origin of the biplot, that is, zero. On the other hand the genotype G1 lies on the origin of the biplot, that is, with zero distance and therefore showed least contribution to GE interaction. Regarding the environments E3 exhibited minimum IPCA 1 score and led to zero interaction, whereas E2 followed by E1 (with IPCA 1 "-") contributed maximum to GE interaction. To understand the relationships between particular genotypes and environments for protein content, AMMI 2 biplot analysis is performed, where IPCA 1 scores are plotted against IPCA 2 scores of the AMMI analysis (Figure 2). The results of this biplot showed the genotype G1 is tightly grouped with environment E3 but contributed least to GE interaction because both lies very close to the origin of the biplot. On the other hand the environments E1 and E2 and rest of the genotypes had maximum GE interaction as indicated by the distances from the origin of biplot.

From the (Figure 3), it is clear that all the genotypes (with IPCA 1 "-") except G1 contributed maximum to GE

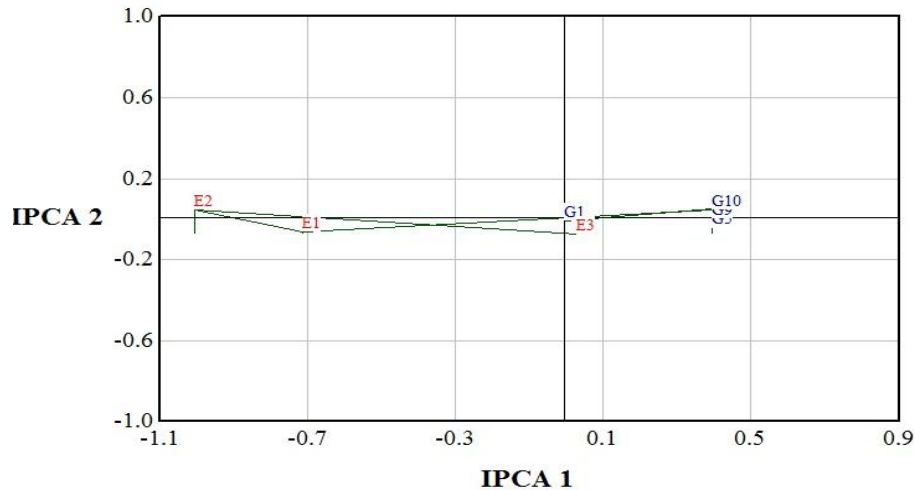


Figure 2. Biplot of the first AMMI interaction (IPCA 2) score (Y-axis) plotted against AMMI interaction (IPCA 1) score (X-axis) for 10 wheat genotypes and 3 environments

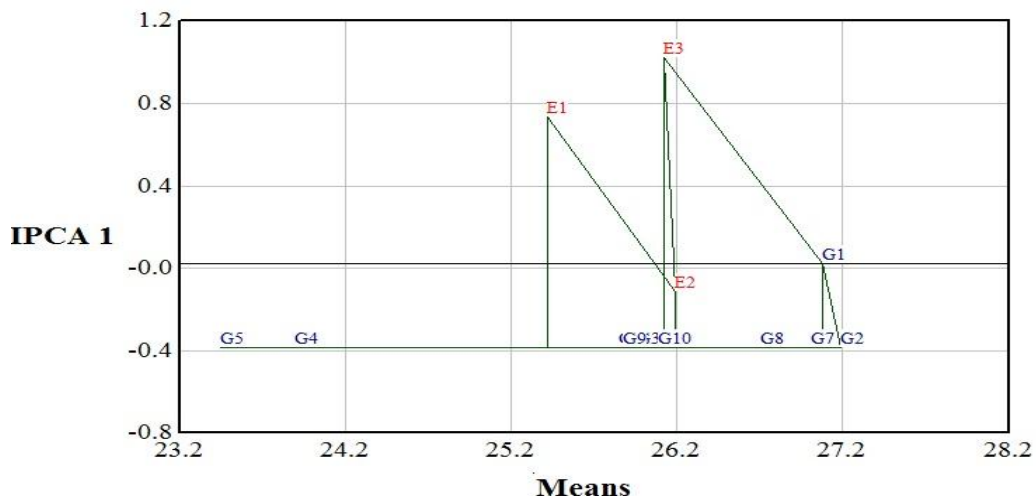


Figure 3. Biplot of the first AMMI interaction (IPCA 1) score (Y-axis) plotted against mean gluten content (%) (X-axis) for 10 wheat genotypes.

interaction. Among them the highest contribution was exhibited by G5 and G4. The environment E2 showed minimum IPCA score and led to zero interaction, whereas the environment E3 followed by E1 contributed maximum to GE interaction as indicated by their distance from the origin of biplot. The AMMI 2 biplot (Figure 4) for gluten content revealed all the genotypes were tightly grouped with E2 and were highly stable to that particular environment. The environments E1 and E3 were separated and showed maximum effect on GE interaction.

It is evident from Table 2 that both protein and gluten content are significantly affected by the wheat varieties under various locations. The highest protein content

(pooled) is exhibited by SKW-489 (13.54%) and SW-1 (13.23%) whereas the lowest protein content was observed in SKW-848 (10.31%). Similarly, highest gluten content (pooled) is observed in SKW-517 (29.65%) and SW-355 (29.14%), while lowest percentage was exhibited by SKW-489 (22.22%). Phenotypic coefficient of variability is higher than genotypic coefficient of variability for both the traits, however, the differences between them is very narrow indicating the lesser role of environment. As the coefficient of variation indicates only the extent of variability, it does not reflect on heritable proportion of variation. Hence, estimation of heritability coupled with genetic advance as per cent of mean permits greater effectiveness for selection by separating out the

Table 2. Protein and gluten content (%) in 13 wheat genotypes under three environments.

Genotypes	Environment I		Environment II		Environment III		Pooled environments	
	Protein content (%)	Gluten content (%)	Protein content (%)	Gluten content (%)	Protein content (%)	Gluten content (%)	Protein content (%)	Gluten content (%)
SKW-848	9.90	26.95	10.64	29.12	10.40	28.62	10.31	28.23
SKW-489	13.13	22.72	13.87	19.55	13.63	24.39	13.54	22.22
SKW-490	12.01	26.49	12.75	28.66	12.26	21.82	12.34	25.65
SKW-514	10.57	24.88	11.31	27.05	11.12	26.55	11.00	26.16
SKW-515	13.56	21.37	12.82	23.54	13.06	23.04	13.14	22.65
SKW-517	11.13	30.93	11.87	28.76	11.63	29.26	11.54	29.65
SKW-519	12.26	25.70	13.00	27.87	12.76	27.37	12.67	26.98
SKW-527	10.81	27.32	11.55	25.15	11.31	28.99	11.22	27.15
SKW-530	11.14	24.31	11.88	26.48	11.64	25.98	11.55	25.59
SKW-531	12.19	23.83	12.93	26.00	12.69	25.50	12.60	25.11
SW-1	12.82	26.89	13.56	29.06	13.32	28.56	13.23	28.17
HS-240	11.96	25.78	12.70	27.95	12.46	27.45	12.37	27.06
SW-355	12.29	29.31	13.03	27.14	12.79	30.98	12.70	29.14
GCV	8.981	9.965	7.490	10.088	7.578	9.784	7.922	8.476
PCV	8.996	9.981	7.502	10.104	8.125	9.799	8.004	8.489
H² (BS)	94.669	98.688	95.689	97.684	86.979	98.691	97.956	98.682
GA % Mean	18.470	20.496	15.406	20.748	14.558	20.124	16.152	17.432

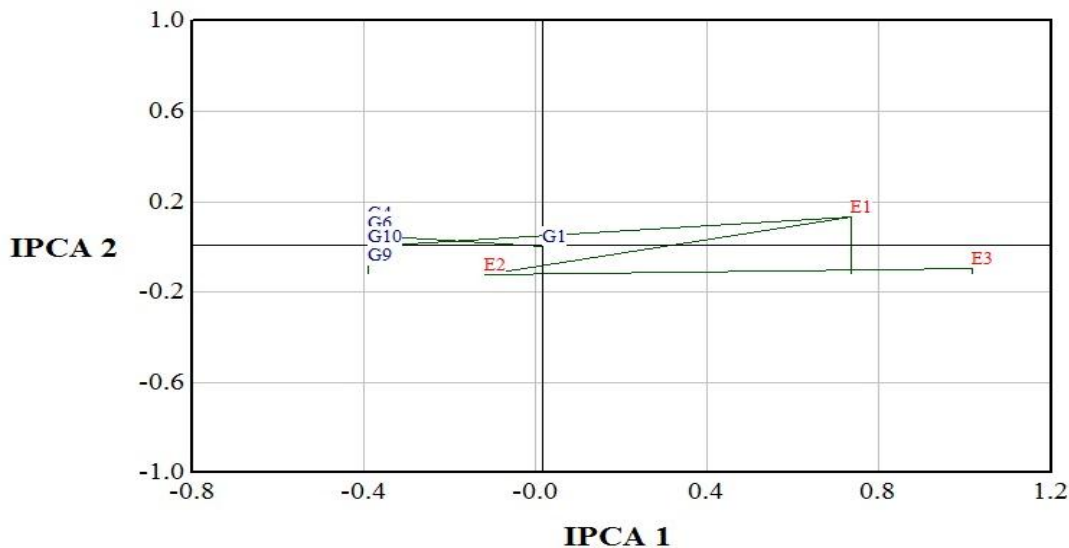


Figure 4. Biplot of the first AMMI interaction (IPCA 2) score (Y-axis) plotted against AMMI interaction (IPCA 1) score (X-axis) for 10 wheat genotypes and 3 environments.

environmental influence from the total variability and thereby allowing accurate selection of a potential phenotype. The results indicated high heritability coupled with high genetic advance for both the traits, thus indicates the predominance of additive genetic variance for these traits. Hence, these characters are amenable for simple selection of superior segregants (Ahmad et al., 2013b). High heritability with high genetic advance was

also reported for protein content by Noorka et al. (2009) and Mueen-ud-Din (2009). Anjum and Walker (2000) observed that dry and wet gluten contents of Pakistani wheats were significantly influenced by cultivars but not by crop years or growth locations. In the present investigation, the AMMI analysis showed that quality traits were highly influenced by genotype, environment and GE interaction. Other studies have reported similar

observations on wheat (Hints et al., 2011). The high protein percentage of SKW-489 and SW-1 indicates their stability for hard wheat products like yeast-leavened bread. The dough made from this type of varieties increases dough strength, resulting in increased loaf volume after baking. High protein levels are related to undesirable cookie texture. Protein content of rest of the genotypes is low to medium in range and characterizes as semi hard wheat. Dough from these varieties will be strong, stretchable, elastic and non-sticky, that is, suitable for un-leavened bread like chapatti. All varieties used in this study are found to have medium to high gluten content. High gluten content observed in SKW-517 and SW-355, interprets strong gluten matrix which may during baking increase the viscosity of cookie dough, which is undesirable because cookie spread is restricted but it is good for bread.

Conclusion

The genotype G1 was tightly grouped with E3 as indicated by their origin on the biplot. All the genotypes except G1 were located in the point farthest from the center of the biplot (PC1), indicating high gluten content, but the length of its PC2 vector exhibits this variety's instability, while G1 was in the center of the biplot exhibiting high stability but lower gluten level than the above mentioned cultivars. However, all the genotypes were tightly grouped with E2 with regard to gluten content and as such highly stable to this particular environment.

Conflict of Interest

The authors have not declared any conflict of interests.

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Full Length Research Paper

Fertigation through trickle and micro sprinkler on flowering characters in cocoa (*Theobroma cacao* L.)

C. Krishnamoorthy^{1*}, K. Rajamani², S. Mekala³ and S. Rameshkumar¹

¹Vanavarayar Institute of Agriculture, Pollachi, Tamil Nadu, India.

²Department of Medicinal and Aromatic Crops, Tamil Nadu Agricultural University, Coimbatore, India.

³Department of Commerce with Computer Applications, Kongu Arts and Science College, Bharathiar University, Tamil Nadu, India.

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An experiment was conducted during 2010 and 2011 to investigate the impact of fertigation on flowering characters of cocoa at Tamil Nadu Agricultural University, India. The study was laid out in randomized block design with thirteen treatment combinations replicated thrice. The study shown that, fertigation with 125% recommended fertilizer dose as water soluble fertilizer through fertigation by micro sprinkler irrigation (T₁₀) recorded the highest number of flower cushions per tree per season (377.6) as against 16.60% increase over the control (314.9) at 0.05% significance. The same treatment (T₁₀) recorded the best in terms of other flowering characters viz., area of pollen grain (504.1 μm^2), equivalent diameter of the pollen grain (27.4 microns), radius of the pollen grain (13.3 microns), pollen output (34.9), pollen viability (89.2%), pollen germination (88.1%) as against 38.39, 23.72, 24.06, 28.65, 17.38 and 36.55% increase over the control (T₁) respectively. Fertigation with 125% recommended fertilizer dose as water soluble fertilizer through fertigation by micro sprinkler irrigation recorded the maximum flowering characters.

Key words: Fertigation, drip, micro sprinkler, flowering characters, *Theobroma cacao*.

INTRODUCTION

India offers considerable scope for cocoa cultivation, production and further development. Though cocoa has been known as the beverage crop even before tea and coffee, it is relatively a new crop to India. Cocoa readily responds to applied fertilizers to meet its nutrient requirements (Armando et al., 2001). Through fertigation methods, nutrients are added to the soil in adequate doses and interval through which qualitative improvement of produce

can also be attained to a larger extent. In India, mostly the cocoa growing farmers are adopted surface irrigation with soil application of fertilizers. This will cause low productivity of less than 1 kg beans per tree per year. Production of quality beans in cocoa (single bean weight of more than 2 g) will enable the farmers to earn more income.

Being a shallow rooted crop, cocoa requires frequent doses of fertilizers coupled with soil moisture to utilize the

*Corresponding author. E-mail: plantdoctorkrishna@gmail.com, Tel: +91 9659498935.

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nutrients more effectively (Noordiana et al., 2007).

Fertigation ensures higher fertilizer use efficiency, besides providing scope for making soil amendments and even biological methods of plant protection (Oliveira et al., 2006). In the fertigation method, fertilizers can be applied throughout the crop growing season in phased manner, in various split doses, in any desired concentration. This is in contrast to the conventional practice where larger amounts of fertilizers are placed on the soil at the beginning of the season in one or very few split doses (Dangler and Locascio, 1990).

Considering the high yield potential in cocoa, nutrient application with due consideration on various crop growth stages viz., vegetative, flowering, pod set, pod development and maturity will help in realizing the potential yield (Krishnamoorthy and Rajamani, 2013). Flowering is cauliflorous and flowering cycle follows certain seasonal patterns. In India, it occurs from January to February with a peak in July to August. Flowering and fruit set are the most critical events occurring after establishment of a crop (Davenport and Nunez-Elisea, 1990).

Fertilization at proper time and proper dose will increase the pod yield (Patel and Rajput, 2000). Flowering in cocoa is determined by multi various factors like genetic factor, environmental factors, age of the tree, plant growth hormones, availability of soil moisture and nutrients as reported by Thondaiman (2011). Application of water soluble fertilizers through micro sprinkler fertigation registered better results in pollen parameters in mango (Davenport and Nunez-Elisea, 1990). Hence, the study was undertaken to evaluate the flowering characters and yield characters of cocoa using different fertigation schedules by drip and micro sprinkler irrigation.

MATERIALS AND METHODS

The geographical location of the experimental site is located at 10° 58' 0" N latitude, 76° 56' 0" E longitude and at an altitude of 258 m above MSL with average annual rain fall of 844 mm. Six year old cocoa trees were selected for the study. The varietal description of cocoa indicated that they were F₁ seedling progenies (Forester type), raised through seedlings (of F₁'s) supplied by Kerala Agricultural University, India. In coconut plantation of thirty year old with spacing of 8 x 8 m, the cocoa plants are intercropped with a spacing of 3 x 3 m (Figure 1). In case of drip irrigation, two emitters were installed with a discharging rate of 8 lph (litres per hour). Two micro sprinklers transmitting at 60 lph micro sprinkler⁻¹ were installed to cover the entire basin. The micro sprinkler type is half sub circle with a height of 30 cm and it has sprinkling capacity of 60 cm area. The venturi was used for mixing of fertilizer with water. The study was laid out in randomized block design with thirteen treatment combinations (Table 1). The experiment having fertilizer application methods at two levels that is, drip and sprinkler and the fertilizer application rates at 3 levels that is, 75, 100 and 125%.

An annual application of 100 g N, 40 g P₂O₅ and 140 g K₂O through the mode of surface irrigation (T₁) is recommended for annual basis per tree in two splits (1st dose in 1st week of April and 2nd dose in 1st week of September). Surface irrigation was carried out once in seven day's interval. The fertilizers were applied through drip and micro sprinkler irrigation system (fertigation) at

weekly intervals for drip and micro sprinkler treatments (T₂ to T₁₃) and the irrigation was carried out once in a day (20 L tree⁻¹ day⁻¹).

Data collection

The observations on flowering characters were recorded as per standard procedures and analyzed statistically. Flowering in cocoa was throughout the year and two peak harvest seasons viz., March to May and September to November were observed. Among these two seasons, March-April (flowering) to July (pod harvest) season is considered as lean cropping period while September (flowering) to December (pod harvest) season is considered as peak cropping period. The geometry of the pollen grains was studied during 2010 and 2011. Pollens grains were collected from freshly dehisced anthers by gently tapping the anthers on glass slides which containing a drop of glycerol. Then cover slips were placed over the pollens and slides were observed under a microscope with the aid of ocular and stage micrometers (Mishra et al., 2006) connected with a computer in ordinary light. The observations were recorded from 50 pollen grains in each treatment and the data were analyzed in Q 500 MC WIN software programme.

RESULTS

In the present study, high variation was noticed in the number of flower cushions per tree and number of flowers per cushion with various fertigation treatments. Application of 125% Resource Description Framework (RDF) as water soluble fertilizer through fertigation by micro sprinkler irrigation recorded the highest number of flower cushions per tree (365.3 and 389.9) during 2010 and 2011 respectively as presented in Table 2. The lowest number of flower cushions per tree was recorded in control (319.4 and 310.4) during 2010 and 2011 respectively. The treatment which received 100% RDF as water soluble fertilizer through fertigation by micro sprinkler irrigation registered more number of flowers per cushion (7.2 and 6.9) during 2010 and 2011 respectively (Table 3).

The trees which received 125% RDF as WSF through fertigation by micro sprinkler irrigation registered the highest pollen grain area (509.8 and 498.3 μm²), equivalent diameter of the pollen grain (27.7 and 27.1 microns) (Table 4), radius of the pollen grain (13.3 and 13.3 μ) during first and second season of 2011.

The highest perimeter of the pollen grain was recorded in the treatment received 125% RDF as water soluble fertilizer through fertigation by micro sprinkler irrigation (98.9 μ) during first season in 2011 (Table 5). During second season, application of 100% RDF as water soluble fertilizer through fertigation by micro sprinkler irrigation registered highest pollen grain value (99.3 μ). Among the several treatments, the treatment which received 125% RDF as water soluble fertilizer through fertigation by micro sprinkler irrigation recorded more pollen output (34.9), pollen viability (89.2%) and pollen germination (88.1%) during 2011 (Table 6).

The control that is, 100% of RDF as surface application with flood irrigation registered minimum pollen tube length (12.8, 60.9, 142.6, 183.0, 262.5, 306.6 μ and 12.4,

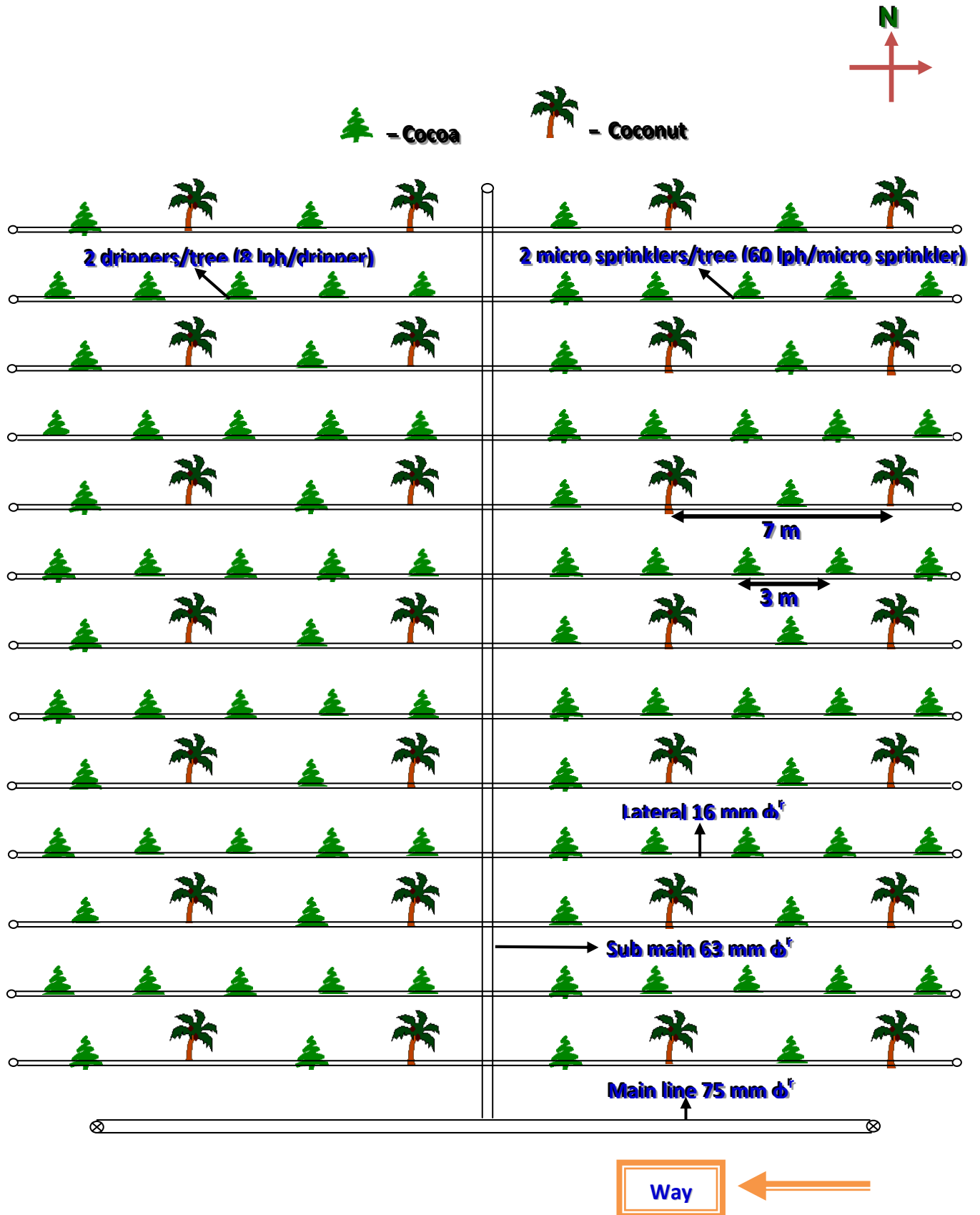


Figure 1. Lay out of drip and micro sprinkler in cocoa.

Table 1. Treatment details of the experiment.

Treatment No.	Dosage	Method of application / irrigation
T ₁	100 % RDF	Surface application + flood irrigation (control)
T ₂	75 % RDF as WSF	Drip
T ₃	100 % RDF as WSF	Drip
T ₄	125 % RDF as WSF	Drip
T ₅	75 % RDF as straight fertilizers	Drip
T ₆	100 % RDF as straight fertilizers	Drip
T ₇	125 % RDF as straight fertilizers	Drip
T ₈	75 % RDF as WSF	Micro sprinkler
T ₉	100 % RDF as WSF	Micro sprinkler
T ₁₀	125 % RDF as WSF	Micro sprinkler
T ₁₁	75 % RDF as straight fertilizers	Micro sprinkler
T ₁₂	100 % RDF as straight fertilizers	Micro sprinkler
T ₁₃	125 % RDF as straight fertilizers	Micro sprinkler

RDF - Recommended dose of fertilizer, WSF - water soluble fertilizer.

Table 2. Effect of drip and micro sprinkler fertigation on number of flower cushions per tree at various seasons for two years.

Treatments	2010			2011			Pooled analysis (2010 and 2011)
	1 st season	2 nd season	Mean	1 st season	2 nd season	Mean	
T ₁	323.6	315.1	319.4	303.5	317.3	310.4	314.9
T ₂	332.9	344.1	338.5	320.6	329.5	325.1	331.8
T ₃	340.0	343.7	341.8	340.2	349.2	344.7	343.3
T ₄	347.9	354.0	350.9	350.8	359.4	355.1	353.0
T ₅	324.1	332.2	328.2	310.4	321.3	315.9	322.0
T ₆	331.3	339.6	335.5	322.6	330.6	326.6	331.0
T ₇	340.5	349.1	344.8	338.2	346.3	342.3	343.6
T ₈	349.9	354.3	352.1	363.1	370.2	366.7	359.4
T ₉	353.4	355.9	354.7	375.2	382.0	378.6	366.7
T ₁₀	360.9	369.7	365.3	384.5	395.2	389.8	377.6
T ₁₁	334.7	344.3	339.5	357.6	366.1	361.9	350.7
T ₁₂	342.9	349.4	346.2	362.3	370.8	366.6	356.4
T ₁₃	350.3	358.9	354.6	367.3	376.8	372.0	363.3
SEd ($P < 0.05$)	5.56	5.68		6.03	6.14		5.82
CD (0.05)	11.48	11.72		12.45	12.66		12.01
CD (0.01)	15.64	15.97		16.96	17.26		16.37

54.9, 130.4, 190.7, 274.1, 290.1 μ) at 1, 5, 10, 15, 20 and 25 h after dehiscence of anther) during first and second season in 2011 (Table 7).

DISCUSSION

Among the different combinations, water soluble fertilizer with micro sprinkler irrigation showed better results. Application of 125 or 100% recommended NPK as water soluble fertilizer through micro sprinkler fertigation produced more number of flower cushions per tree and number of flowers per cushion. In general, the number of

flower cushions per tree in the first year (2010) was lower as compared to second year (2011). The flower cushions in cocoa are spread over the trunk and main branches, the number is influenced more due to the soil nutrient; climatic conditions existed in the previous year. The fertigation treatments imposed in the first year would have influenced emergence of new flower cushions only in the second or later years. Because the trees perform better in second year with the increased flowering is in line with findings of Mongi-Zekri and Koo (2003).

The better photo-assimilates and hormonal balance would have improved the sink strength of trees treated with cent per cent RDF through micro sprinkler fertigation

Table 3. Effect of drip and micro sprinkler fertigation on number of flowers per cushion at various seasons for two years.

Treatments	2010			2011			Pooled analysis (2010 and 2011)
	1 st season	2 nd season	Mean	1 st season	2 nd season	Mean	
T ₁	4.4	4.8	4.6	4.6	4.7	4.6	4.6
T ₂	5.1	5.2	5.2	5.0	5.0	5.0	5.1
T ₃	5.3	5.6	5.5	5.4	5.5	5.5	5.5
T ₄	5.9	5.4	5.7	5.6	5.3	5.5	5.6
T ₅	4.9	5.1	5.0	5.2	4.9	5.1	5.0
T ₆	4.7	4.9	4.8	5.0	5.1	5.1	4.9
T ₇	5.1	5.0	5.1	4.9	5.0	4.9	5.0
T ₈	6.0	6.0	6.0	6.5	5.7	6.1	6.1
T ₉	7.4	6.9	7.2	7.0	6.8	6.9	7.0
T ₁₀	7.2	7.0	7.1	6.9	6.6	6.8	6.9
T ₁₁	5.4	6.1	5.8	6.4	6.3	6.4	6.1
T ₁₂	6.4	6.2	6.3	6.9	6.6	6.8	6.5
T ₁₃	6.1	5.8	5.9	6.6	6.4	6.5	6.2
SEd (<i>P</i> < 0.05)	0.12	0.11		0.12	0.11		0.12
CD (0.05)	0.25	0.23		0.25	0.23		0.24
CD (0.01)	0.34	0.31		0.34	0.32		0.33

Table 4. Effect of drip and micro sprinkler fertigation on area and equivalent diameter of the pollen grain at various seasons.

Treatments	Area of the pollen grain (μm^2)			Equivalent diameter of the pollen grain (Microns)		
	1 st season	2 nd season	Mean	1 st season	2 nd season	Mean
T ₁	309.5	311.6	310.6	20.3	21.6	20.9
T ₂	337.6	348.9	343.2	21.7	21.9	21.8
T ₃	395.1	438.9	417.0	23.7	23.8	23.7
T ₄	451.6	386.3	418.9	24.7	25.4	25.0
T ₅	317.1	322.6	319.8	21.0	21.6	21.3
T ₆	351.3	364.7	358.0	22.0	22.5	22.3
T ₇	393.1	400.7	396.9	23.3	23.9	23.7
T ₈	457.3	422.8	440.1	25.0	24.9	24.9
T ₉	497.5	486.1	491.8	26.3	26.6	26.5
T ₁₀	509.8	498.3	504.1	27.7	27.1	27.4
T ₁₁	452.5	473.7	463.1	24.7	24.3	24.5
T ₁₂	464.4	459.6	462.0	25.0	25.0	25.0
T ₁₃	496.9	478.6	487.7	26.0	25.9	25.9
SEd (<i>P</i> < 0.05)	9.18	8.74		0.43	0.42	
CD (0.05)	18.95	18.04		0.89	0.88	
CD (0.01)	25.82	24.59		1.22	1.19	

(subjected to higher levels of available nutrients through fertigation) through acceleration of megal and microsporogenesis and differentiation of axillary buds into reproductive ones. Higher level of N, P and K resulted in rapid flower production than lower levels as reported by Takahashi et al. (1993).

Application of water soluble fertilizers through micro sprinkler fertigation registered better results in pollen

parameters like area, equivalent diameter, radius, perimeter, pollen output, pollen viability, pollen germination and pollen tube length. These might be due to enhanced level of auxin-like substances which would have been triggered by split application of WSF through micro sprinkler fertigation. High levels of auxin-like substances promote the flowering parameters either by nullifying the effect of GA₃ (or) by decreasing the permeability of cell membrane, particularly

Table 5. Effect of drip and micro sprinkler fertigation on radius and perimeter of the pollen grain at various seasons.

Treatments	Radius of the pollen grain (Microns)			Perimeter of the pollen grain (Microns)		
	1 st season	2 nd season	Mean	1 st season	2 nd season	Mean
T ₁	10.0	10.1	10.1	75.0	77.3	76.1
T ₂	10.2	10.2	10.2	79.3	78.7	78.9
T ₃	11.9	11.8	11.9	82.8	81.4	82.1
T ₄	12.1	11.9	12.1	85.8	82.9	84.4
T ₅	10.2	10.2	10.2	77.3	77.7	77.5
T ₆	11.2	11.1	11.1	79.5	78.2	78.9
T ₇	11.7	11.5	11.6	82.8	83.9	83.4
T ₈	12.4	12.2	12.3	93.7	89.7	91.7
T ₉	13.2	13.1	13.1	97.8	99.3	98.6
T ₁₀	13.3	13.3	13.3	98.9	96.2	97.5
T ₁₁	12.4	12.6	12.5	87.4	86.5	86.9
T ₁₂	12.5	12.9	12.7	96.0	97.7	96.9
T ₁₃	12.9	13.1	12.9	97.8	95.6	96.7
SEd ($P < 0.05$)	0.22	0.22		1.60	1.58	
CD (0.05)	0.45	0.45		3.30	3.27	
CD (0.01)	0.61	0.61		4.50	4.45	

Table 6. Effect of drip and micro sprinkler fertigation on pollen output (numbers), pollen viability (%) and pollen germination (%) at various seasons.

Treatments	Pollen output / 10 anthers			Pollen viability (%)			Pollen germination (%)		
	1 st season	2 nd season	Mean	1 st season	2 nd season	Mean	1 st season	2 nd season	Mean
T ₁	25.4	24.5	24.9	72.1	75.3	73.7	52.5	59.4	55.9
T ₂	27.0	26.3	26.7	74.7	76.0	75.4	63.1	74.4	68.8
T ₃	29.3	28.5	28.9	76.5	77.7	77.1	71.3	66.8	69.0
T ₄	30.5	30.7	30.6	80.2	79.5	79.8	76.9	79.0	78.0
T ₅	30.9	30.6	30.8	74.9	76.9	75.9	54.6	70.6	62.6
T ₆	30.9	31.0	30.9	78.4	75.4	76.9	78.5	72.3	75.4
T ₇	31.0	31.9	31.5	76.9	78.0	77.5	75.6	74.6	75.1
T ₈	32.3	31.0	31.7	79.9	81.9	80.9	82.0	80.9	81.5
T ₉	33.8	33.6	33.7	88.7	89.7	89.2	90.9	84.6	87.8
T ₁₀	34.5	35.4	34.9	90.5	87.8	89.2	88.7	87.4	88.1
T ₁₁	30.6	31.1	30.9	82.3	84.4	83.4	79.3	81.4	80.4
T ₁₂	32.0	33.2	32.6	86.3	82.5	84.4	86.0	78.9	82.5
T ₁₃	33.4	33.5	33.4	86.0	83.2	84.6	81.7	93.2	87.5
SEd ($P < 0.05$)	0.54	0.55		1.39	1.35		1.61	1.47	
CD (0.05)	1.11	1.14		2.86	2.79		3.33	3.03	
CD (0.01)	1.51	1.56		3.89	3.80		4.54	4.13	

the plasmalemma (Eyheraguibel et al., 2008). Davis et al. (1991) also supported this by stating that amino-succinamic acid (B 995) increases the membrane permeability of fruits. Many plant growth substances develop a strong physical association with lecithin and have direct influence on cell membrane functions towards reproductive parts growth as opined by Keith (2006).

Conclusion

Fertigation studies on cocoa through micro sprinkler irrigation with a dose of 100 or 125% RDF as water soluble fertilizer (WSF) has shown increase in flowering characters like number of flower cushions per tree (377.6), number of flowers per cushion (7.03), equivalent diameter of the

Table 7. Effect of drip and micro sprinkler fertigation on pollen tube length (Microns).

Treatments	Hours after dehiscence of anther											
	1 st season						2 nd season					
	1	5	10	15	20	25	1	5	10	15	20	25
T ₁	12.8	60.9	142.6	183.0	262.5	306.6	12.4	54.9	130.4	190.7	274.1	290.1
T ₂	14.7	76.9	153.3	197.3	273.9	332.8	14.1	72.3	133.4	196.9	283.0	301.8
T ₃	14.9	78.6	151.0	216.3	280.4	323.4	14.2	69.3	149.7	208.1	282.4	310.4
T ₄	15.0	81.5	143.5	210.6	281.6	318.8	16.8	59.7	150.3	206.9	285.3	320.5
T ₅	14.7	61.9	164.7	199.8	276.7	309.4	14.3	56.6	135.0	204.6	290.5	306.2
T ₆	14.2	61.3	148.7	200.5	284.8	312.8	15.4	62.8	140.0	226.4	280.6	304.6
T ₇	15.2	70.3	162.8	216.9	289.1	310.7	15.6	71.5	138.9	214.3	291.9	312.5
T ₈	19.2	96.8	168.2	240.4	314.9	336.1	18.0	76.6	178.2	229.6	301.8	330.9
T ₉	24.3	91.7	174.1	238.2	300.4	347.2	24.8	108.3	182.8	240.8	318.3	352.4
T ₁₀	19.2	100.8	170.0	233.6	306.2	341.1	17.4	94.6	172.8	238.9	316.7	368.7
T ₁₁	18.9	120.4	169.3	220.6	292.6	325.8	18.2	83.0	170.4	231.6	311.0	331.7
T ₁₂	20.6	98.1	178.8	219.7	314.0	348.7	20.7	99.7	174.3	244.8	308.9	354.1
T ₁₃	22.6	100.4	183.9	234.5	299.8	354.9	26.9	99.1	186.9	236.8	313.5	360.7
SEd ($P < 0.05$)	0.42	2.13	2.81	3.81	4.86	5.47	0.47	1.99	3.13	3.89	4.91	5.69
CD (0.05)	0.87	4.39	5.81	7.86	10.03	11.28	0.96	4.11	6.45	8.02	10.14	11.75
CD (0.01)	1.19	5.98	7.91	10.71	13.67	15.38	1.31	5.60	8.80	10.93	13.81	16.01

pollen grain (27.4 μ), radius of the pollen grain (13.3 μ), pollen output (34.9), pollen viability (89.2%), pollen germination (88.1%). In conclusion, fertigation with 100 or 125% RDF as WSF recorded the maximum flowering parameters in cocoa.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Production of particleboards from licorice (*Glycyrrhiza glabra*) and European black pine (*Pinus Nigra* Arnold) wood particles

Cengiz GULER

Department of Forest Industrial Engineering, Faculty of Forestry, Wood Science and Technology, Duzce University, 81620 Duzce, Turkey.

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In this study particleboards were manufactured from mixtures of licorice (*Glycyrrhiza glabra*) and European Black Pine (*Pinus nigra* Arnold) wood particles at several ratios. The suitability of licorice (*Glycyrrhiza glabra*) chips for particleboard production was examined. Urea formaldehyde resin was used as a binder in 3-layers particleboards. The manufactured boards were tested according to European (EN) standards. The licorice and European Black Pine wood particles were mixed at ratios 25, 50, 75 and 100% respectively. Produced panels were tested for certain mechanical and physical properties. Experimental results indicated that increase in licorice chips in the mixture generally diminished the mechanical and physical properties. The produced boards can be utilized for general purposes as well as furniture for interior environments and the results obtained showing that licorice could be an alternative raw material for particleboard industry.

Key words: Particleboard, licorice (*Glycyrrhiza glabra*), physical and mechanical properties, European black pine (*Pinus nigra*).

INTRODUCTION

In recent years the utilization of agricultural waste and annual fibers for particleboard or other composite board production is going popular. Particleboard is one of the most important products of forest products industry known. Throughout the years, the production cost of particle board has increased while the quality of product reduced.

Agricultural waste materials and annual plants have become alternative raw materials for the production of particleboard or fiber composite materials. While many

industries utilise forest products as a raw material and uneconomical use of these resources cause extinction of forest. It is really necessary to find alternative raw material source in order to reduce forest consumption. For this reason it is important to study suitability of annual plants fibres for particleboard production. This will aid protection of environment and as well as development of environmental friendly technologies. The most frequently referred alternative non-wood materials are flax, bagasse, hemp, reed and cereal straws such as rice and

E-mail: cengizguler@duzce.edu.tr

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Table 1. Properties of the UF adhesive.

Properties	UF ^a
Solid (%)	55±1
Density (g/cm ³)	1.20
pH	8.5
Viscosity (cps)	160
Ratio of water tolerance	10/27
Reactivity	35
Free formaldehyde (%)	0.15
33% NH ₄ Cl content (max, %)	1
Gel point (100°C, sec.)	25-30
Storage time (25°C, max day)	90
Flowing point (25°C, sec.)	20-40

^aUrea formaldehyde.

Table 2. Experimental design.

Board type ^a	Raw material	
	Licorice roots	Pine chips
A	100	-
B	75	25
C	50	50
D	25	75
E	-	100

^aThe density of the boards made from licorice and European Black Pine wood chips was 0.70 g/cm³.

wheat straw (Younquist et al., 1994). Today, chemical pulp and panel products using wheat straw and other crop residues are being commercially manufactured in a number of countries including Turkey (Copur et al., 2007). There is still an outgoing research interest to find alternative sources of raw materials for composite manufacturing.

In recent studies; sugarcane bagasse (Silva et al., 2014), cotton stalks (Guler and Ozen, 2004), cotton carpel (Alma et al., 2005), hazelnut husk (Guler et al., 2009), Oil palm (Ratnasingam et al., 2008), bamboo chips (Papadopoulos et al., 2004), kenaf core and kenaf stalks (Xu et al., 2004; Kalaycioglu and Nemli, 2004), date palm branches (Nemli et al., 2001), Eggplant stalks (Guntekin and Karakus 2008), wheat straw and corn pich (Wang and Sun, 2002), peanut hull (Guler and Buyuksari, 2011), sunflower stalks (Khristova et al., 1998; Bektas et al., 2005; Guler et al. 2006) and pepper stalks (Guntekin et al., 2008) have been investigated.

The problems of the industrial usage of agricultural residues in particleboard industry refer to the high cost of collecting, transporting, and storing of the residue materials. Some of these problems could be overcome by building local, small scale mills, close to the rural areas.

In Turkey licorice, sunflower, rice, wheat, straw, sugar cane and cotton are produced for vegetable oil fiber or food industries. The waste products of these agricultural plants are consumed for animal feed fertilizer or heat production.

One of the agricultural residues, licorice (*Glycyrrhizaglabra*L.), is produced by former USSR, Spain, Turkey, Syria, Iraq and Afghanistan. Most of the commercial supply comes from wild sources and only a limited amount is cultivated. In addition to its medicinal use, licorice has been industrially utilized in chocolates, beverages and tobacco (Copur et al, 2007). However, in utilization of licorice fibers, there is almost no literature in the particleboard industry.

Eastern Anatolia in Turkey is covered with licorice plant about 4 million acres. Utilization of licorice in particleboard industry might produce economic benefits and contribute to environmental sustainability. Therefore, this study focuses on the potential use of licorice roots as a source of board making raw material.

The possible utilization of licorice in particleboard industry might produce economic benefits and contribute to environmental sustainability. Therefore, this study focuses on the potential use of licorice roots as a source of panel making raw material.

MATERIALS AND METHODS

Licorice roots obtained from the Mediterranean region of Turkey were cleaned from dust and dirt. Licorice roots and pine wood were coarsely chopped. The particles were sifted by utilizing a horizontal position sieve. 3 to 1.5 mm average size particles were used in the core section of particleboard while 1.5 to 0.8 mm average size particles were utilized in the shell layers.

All particles used in this study were dried at 100 to 110°C in a technical oven until 3% MC is reached. Urea formaldehyde (UF) resin is the most common applied on wood panels (Silva et al., 2013). The resin was applied at 9% for the core layer and 11% for the shell layers based on oven dry weight. The properties of the UF resin are given in Table 1. As a hardener, 33% of ammonium chloride solution was used for all of the UF resin boards. Panels with a target density of 0.70 g/cm³ were manufactured using 0, 25, 50, 75 and 100% licorice chips in the mixture. Experimental design are given in Table 2. The dimensions of the produced particleboards were 50×50×2 cm in pressing and after edge trimming the final dimensions of the particleboards were 47×47×2 cm. The pressing conditions were as follows; press temperature: 150°C; press time: 7 min; pressure: 2.4-2.6 N/mm² and production parameters of boards used in this study are shown in Table 3.

Prior to testing, the boards produced were conditioned at 65 ± 5% Relative Humidity (RH) and 20 ± 1°C in according to TS 642-ISO 554 (1997) Hardboard Method. The samples were cut from the experimental boards to determine some physical and mechanical properties in accordance with TS-EN 310 (1999), TS-EN 317 (1999), TS-EN 319 (1999), and TS-EN 312 (2012) standards. Mechanical and physical properties were tested 10 and 20 specimens respectively.

The analysis of variance (ANOVA) was applied using randomized complete design to find out the statistical differences in the physical, mechanical and dimensional properties of the particleboard. A Duncan multiple range test at 5% level of probability was conducted to study the significance of the

Table 3. Production parameters of particleboards.

Parameter	Value
Press temperature (°C)	150
Pressing time (min)	7
Peak pressure (N/mm ²)	2.4-2.6
Thickness (mm)	20
Dimensions (mm)	500x500
Outer layer (Whole of board %)	35
Middle layer (Whole of board %)	65
Number of board for each type	2

Table 4. The mechanical properties of particleboards made from licorice root and European Black Pine and the test results of ANOVA and Duncan's mean separation tests.

Mechanical properties	Board type	Mean ^a	Std. deviation	Std. error	X _{Min} ^b	X _{Max} ^c	p ^d
MOR (N/mm ²)	A	12.02 ^p	0.986	0.916	9.36	13.32	*
	B	13.50 ^s	1.589	0.758	10.89	15.77	*
	C	13.83 ^{su}	1.649	0.980	10.90	16.58	*
	D	14.78 ^u	2.214	0.654	12.37	18.24	*
	E	16.42 ^v	1.485	0.508	13.41	19.65	*
MOE (N/mm ²)	A	2142.21 ^p	185.532	61.576	1983.00	2286.00	*
	B	2242.36 ^{ps}	145.541	58.196	1958.00	2372.00	*
	C	2358.16 ^{su}	198.588	68.617	2101.00	2680.00	*
	D	2487.52 ^u	212.122	75.357	2125.00	2784.00	*
	E	2582.62 ^v	202.310	48.986	2234.00	2625.00	*
IB (N/mm ²)	A	0.33 ^p	0.154	0.080	0.23	0.48	*
	B	0.45 ^s	0.109	0.089	0.35	0.54	*
	C	0.49 ^u	0.106	0.071	0.32	0.65	*
	D	0.52 ^{uv}	0.154	0.045	0.35	0.68	*
	E	0.55 ^v	0.136	0.023	0.41	0.69	*

^a, Mean values are the average of 10 specimens; ^b, minimum value; ^c, maximum value; ^d, significance level of 0.01 (for ANOVA); ^{p,s,u,t,v}, values having the same letter are not significantly different (Duncan test). MOR, Modulus of rupture; MOE, modulus of elasticity; IB, internal bond strength.

differences among species using Statistical Analysis System.

RESULTS AND DISCUSSION

Table 4 indicated results for the mechanical properties of the produced particleboard. Results indicated that licorice root ratios used in particleboard production significantly affect modulus of rupture, modulus of elasticity and internal bond of the product. The highest MOR and MOE values of 16.42 and 2582.62 N/mm² were observed when only pine wood was utilized in the manufacture of the particleboard (E), respectively. On the other hand, the lowest MOR and MOE values of 12.02 and 2142.21 N/mm² were obtained produced panels with licorice root ratio of 100%. It can be easily seen that mechanical

properties of particleboards were reduced with the increase of licorice root ratio in the panels. The standard method TS-EN 312 (2012) recommends a minimum modulus of rupture (MOR) and modulus of elasticity values of 11 and 1600 N/mm² for the particleboards manufactured for general propose-use as well as furniture for interior environments respectively. The findings in this study showed that all panel types particleboards met the minimum requirement for MOR and MOE.

In the case of IB, similar to the other mechanical properties, the highest IB value of 0.55 N/mm² was observed with the particleboard produced using 100% pine wood. The lowest IB value of 0.33 N/mm² was obtained produced panels with licorice ratio of 100%. Figure 1 shows the IB strengths of the particleboards.

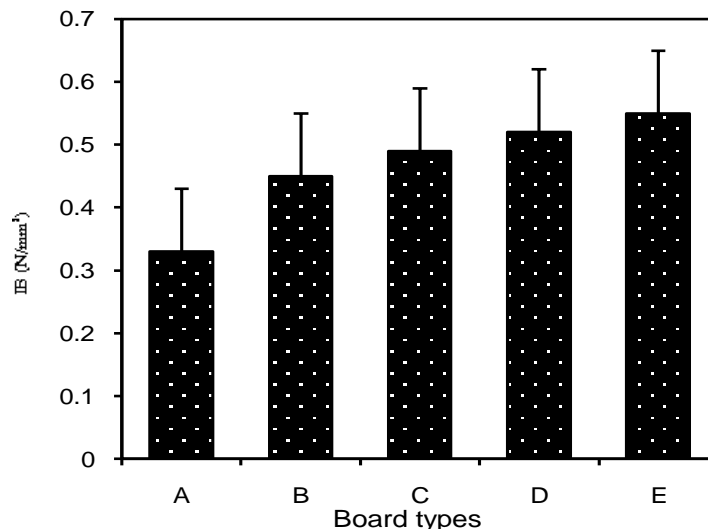


Figure 1. The internal bond strength of the particleboards.

Table 5. Thickness swelling (TS) and water absorption (WA) test results of ANOVA and Duncan's mean separation tests of particleboards produced from licorice root and European Black Pine wood particles.

Physical properties	Board type	Soaking time (2 and 24 h)	Mean (%) ^a	Std. deviation	Std. error	X _{Min} ^b	X _{Max} ^c	p ^d
Thickness swelling (TS)	A	2	17.12 ^P	1.118	1.012	12.36	24.26	*
	B	2	15.54 ^S	1.251	1.131	11.95	25.30	*
	C	2	15.14 ^S	0.546	1.560	09.52	23.02	*
	D	2	14.21 ^U	0.654	0.658	22.31	24.11	*
	E	2	13.25 ^V	1.284	1.092	21.48	22.54	*
	A	24	20.19 ^P	1.451	0.100	12.21	24.99	*
	B	24	20.68 ^P	1.547	0.481	12.14	22.36	*
	C	24	19.32 ^{PS}	1.874	0.222	14.15	23.20	*
	D	24	18.36 ^S	1.687	0.130	27.43	28.50	*
	E	24	17.65 ^U	1.548	0.461	13.52	20.23	*
Water absorption (WA)	A	2	48.25 ^P	2.487	1.018	39.41	57.65	*
	B	2	44.26 ^S	2.683	1.985	30.31	55.62	*
	C	2	38.39 ^U	2.248	1.358	28.43	56.68	*
	D	2	38.01 ^{UV}	2.658	1.654	32.57	42.25	*
	E	2	39.07 ^V	2.952	1.462	28.50	48.74	*
	A	24	59.69 ^P	1.254	0.100	42.99	72.48	*
	B	24	59.26 ^{PS}	2.547	0.654	45.58	85.64	*
	C	24	57.29 ^S	2.654	0.588	51.39	88.40	*
	D	24	55.25 ^U	1.852	0.165	43.14	75.17	*
	E	24	54.90 ^{UV}	1.927	0.187	40.81	74.40	*

^a, Mean values are the average of 20 specimens; ^b, minimum value; ^c, maximum value; ^d, significance level of 0.01 (for ANOVA); ^{p,s,u,v}, values having the same letter are not significantly different (Duncan test).

Particleboards met the IB requirement of 0.35 N/mm² for general purpose end-use as well as furniture for interior environments expect for A type. This finding is compatible with previous results reported in literature

(Goker et al, 1993, Bektas et al., 2005; Guler et al., 2006).

Table 5 shows the results of ANOVA and Duncan's mean separation tests for water absorption and thickness

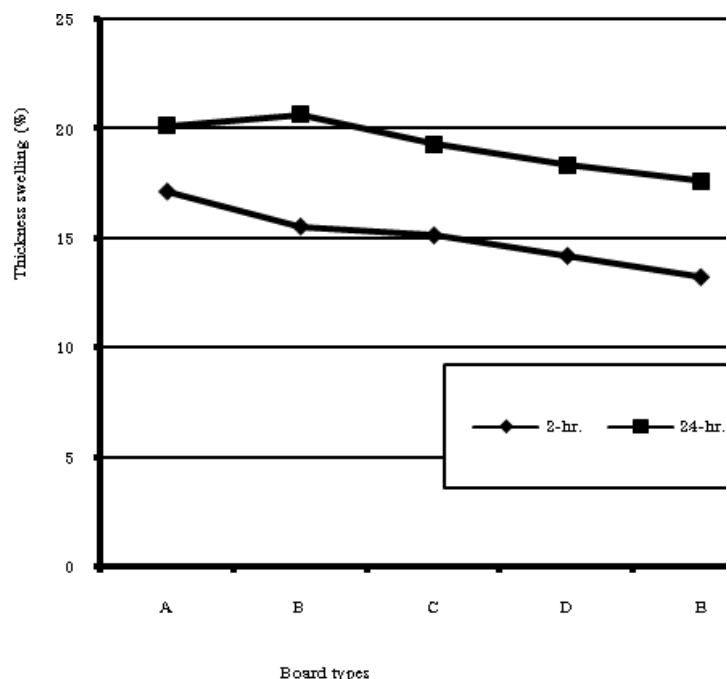


Figure 2. The effect of two different soaking times on thicknesses swelling of board types.

swelling for 2 and 24 h water immersion times. The highest water absorption (48.25 and 59.69%) and thickness swelling (17.12 and 20.19%) were observed with the particleboard (A) having 100% licorice root in the mixture for 2 and 24 h water immersion times, respectively. A diagram of the effect of two different soaking times on the TS of the boards is shown in Figure 2. Increase in licorice root percentage in the mixture resulted in a higher thickness swelling and water absorption for particleboards produced using licorice root and wood chip mixtures. The observed results indicated that the particleboards (A, B, C, and D) including licorice root in the mixture resulted in higher thickness swelling (more than 14%) required by TS EN 312 (2012) Standard. The values obtained with licorice root were found to be comparable with other agricultural residues investigated earlier in the literature: thickness swelling and water absorption values of 17 and 60% for tobacco, 29 and 70% for tea leaves (Kalaycioglu 1992), and 30 and 72% for cotton stalks (Guler and Ozen, 2004) were reported for 24 h water immersion time, respectively. Utilizing water repellent chemicals, such as paraffin in the production may improve these properties.

Conclusions

This study investigated the possibility of using licorice root and European Black Pinewood mixture to manufacture three-layer particleboards. For this purpose,

the mixture of particles produced from licorice root and black pine wood at certain concentrations (0, 25, 50, 75 and 100%) have been used. Five types of panels are produced and their mechanical and physical properties are evaluated. The results indicated that it is possible to produce particleboards from licorice root alone and from the mixture of licorice root and wood chips by using urea-formaldehyde adhesives. The produced boards can be utilized for general purposes as well as furniture for interior environments and the results obtained show that licorice could be an alternative raw material for particleboard industry. Even though increase in the concentration of licorice root particles in composite matrix reduces both the physical and mechanical properties of the particleboard, almost all the studied properties (that is, modulus of rupture, modulus of elasticity, internal bond strength) of the produced panels comply with the minimum requirements in standards for general grade particleboards with the exception of thickness swelling and water absorption. As there are no hydrophobic additives used in these panels, these properties can be improved by the utilization of hydrophobic materials in the matrix. Overall, this study showed that licorice root could be utilized as a raw material in particleboard production by itself or in combination with European Black Pine wood mixture.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

An enhanced requirements elicitation framework based on business process models

Rana Yousef* and Tamara Almarabeh

Computer Information Systems Department, KASIT, the University of Jordan, Jordan.

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Requirements elicitation is a central and critical activity in the requirements engineering process. Completeness is among the most difficult challenges facing requirements engineers. Missing requirements is one of the major causes of software failure; they often result from the lack of anticipation of all possible relations between elements of the system-to-be. In this paper, we propose a requirements elicitation framework which starts with an organization's business process models and buildsthe system's CRUD matrix. This matrix provides all possible relationships between entities and functions of the system in order to capture all possible requirements of the system. The generated relationships between entities and functions provide analysts with the required prompts to ask potential users/stakeholders during interviews to ensure encompassing all questions. The new framework is demonstrated using a real case study; the Cancer Care and Registration in Jordan.

Key words: Requirements elicitation, essential business entities, requirements completeness, missing requirements, CRUD matrix, business process models.

INTRODUCTION

Requirements elicitation represents the set of activities involved in discovering what the system requirements are, including: The identification of all stakeholders of the system, analysis of the problem application domain, the system's operating environment and of the customers' organizational and business environment (Damian, 2000). Improper capturing of system requirements is the major factor in the failure of most software projects.

Requirements are most commonly written in natural language or represented in semi-formal modelling representations. However, a written natural language requirement is error prone and vague leading to

inherent imprecision, such as ambiguities, incompleteness and inaccuracy (Kamalrudin et al., 2011). Completeness of requirements is of major significance in software engineering and can have a major impact on testing, formal verification, robustness and safety of software. Missing requirements are reported as one of the major causes of software failure (Alrajeh et al., 2012; Cheng et al., 2007); they often result from the lack of anticipation of all possible relations between elements of the system-to-be. In this paper, we propose a new requirements elicitation framework which helps anticipate all possible relations between entities and functions for the system starting

*Corresponding author. E-mail: rana.yousef@ju.edu.jo

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with BPMs and building the system's CRUD matrix. This matrix provides all possible relationships between entities and functions in order to capture all possible requirements of the system. So, the ultimate goal is to capture all possible user requirements of a system and reduce the number of missing requirements as much as possible.

The new framework is proposed after providing an overview of the current requirements elicitation techniques and discussing challenges facing requirements engineers during the requirements elicitation phase. The framework is then demonstrated using a real case; the Cancer Care and Registration in Jordan (CCR).

REQUIREMENTS ELICITATION TECHNIQUES

Here, we provide a brief description of the most popular requirements elicitation techniques; these are interviews, questionnaires, observations, brainstorming, focus group, prototyping, document analysis, joint application development and interface analysis (Kotonya et al., 1998; Hickey et al, 2002; Hickey et al, 2003; Parvianen et al, 2007; Wieggers, 2013; Dyba, 2013; Iqbal, 2014).

Interviews

Interviews are the commonly used and most popular method for requirements elicitation (Kotonya et al., 1998; Sampaio et al., 1996). First you identify the range of people who are involved as potential users/stakeholders of the system. This extends the range of viewpoints that will eventually feed into your proposed system. However, the completeness and correctness of the elicited requirements relies to a great extent to the way the interviewer asks the questions. Our proposed framework helps the interviewer in phrasing questions through identifying all possible relationships between business entities and functions, and hence allows him/her to include all possible questions within the system boundaries and reduce the chance of missing any question and consequently reducing the chance of missing any requirement.

Questionnaires

Questionnaires can be used to get responses from a larger group of people than can be handled in interviews. Eliciting requirements through questionnaires involves asking individuals to respond to a fixed set of questions, often by indicating their preferred option from a list of alternative responses to each question. The correctness and completeness of requirements elicited through questionnaires also depend on the way questions are presented.

Observation

Observation is the approach used to collect requirements by observing how people do their work. This method is used to collect requirements when users are too busy to be involved in interviews. Observation helps elicit implicit requirements that interviews can not reveal. However, it is time consuming and may not work if the current process involves intellectual work or work that is not easily observable.

Brainstorming

Brainstorming is a technique used to generate new ideas and find the solution to a specific issue (Pfleeger et al., 1998; Robertson et al., 1998; Wilson, 2006). This is conducted as a conference with 6 to 10 members from different departments and domain experts.

Brainstorming includes choosing a topic or a problem, and then drawing up and discussing different solutions. This method helps the group members to share their experiences and creativity to find the best solution. It's also helpful in eliciting requirements in a relatively short time period, but depends on the member's creativity.

Focus group

Focus group is getting a group of people together to discuss a problem area, preferably with some sort of 'stimulus material' relating to the topic under consideration. The focus group technique can be traditional where members gather in the same physical room or can be an online focus group which allows members to be located remotely while participating.

This technique is effective for learning people's attitudes, experiences and desires; it also gives the ability to ask questions and creates an environment where participants can consider their personal view in relation to other perspectives. However, this method suffers from several problems; if the group is too homogenous the group's responses may not represent the complete set of requirements. It is also difficult to schedule the group for the same date and time. Finally, the collected data (what people say) may not be consistent with how people actually behave.

Prototyping

A Prototype is the representation or visualization of the actual system parts (Andriole, 1994; Kotonya et al., 1998; Robertson et al., 1998). Prototyping aims to uncover and visualize interface requirements before designing or developing the application. But the process of gathering requirements is limited because it is difficult to discover

the users/stakeholders expectations without providing some model. A prototype may lead users to set unrealistic expectations of the delivered system's performance, reliability and usability characteristics.

Document analysis (requirements reuse)

Document analysis is used to gather details of the "As Is" environment such as existing business rules, entities, and attributes that need to be included in a new system or need to be updated for the current system. It is used to elicit requirements of an existing system by reviewing the documentation of such system. Reviewing the documentation helps understand the current situation and begin to formulate the questions to ask stakeholders to gather additional requirements. Documents analysis is the first step to prepare for interviews or other interaction based elicitation techniques. One of the drawbacks of this method is that documentation can be outdated so it is important to confirm that what you are reviewing is the most current. Reviewing existing documentation can also be a time consuming process. Accordingly, our proposed framework, helps reduce the time required to analyse the existing system through a semi-automated approach of analysing the set of business process models available for the as-is system.

Requirements workshop (joint application development, JAD)

A requirements workshop, also referred to as joint application development (JAD), is a structured requirements elicitation method (Maiden et al., 1996). It is similar to brainstorming, except that stakeholders are the ones who are involved in the discussion of the proposed system. Key stakeholders should be carefully selected for a short, intensive period of time (typically one or few days) (Maiden et al., 1996). Involving too many participants can slow down the workshop process thus negatively impacting the schedule. Conversely, collecting input from few participants can lead to overlooking requirements that are important to users, or to specifying requirements that do not represent the needs of majority of the users.

Interface analysis

An interface is a connection between two components. Most systems require one or more interfaces with external parties, systems or devices. Interface analysis is initiated by project managers and analysts to reach agreement with the stakeholders on what interfaces are needed. Interface analysis helps to clarify the boundaries of the system. Scenarios are generally used to clarify

what the stakeholders need in each interaction. Use cases are the basic guidelines for the scenario models. A thorough interface analysis will describe the purpose of each interface involved and elicit high-level details about it, and outline its content. This type of elicitation is essential for software solutions which involve applications interacting with one another and/or users interacting with applications. The disadvantage of this technique is that it does not provide an understanding of the business process since this technique only exposes the inputs, outputs and key data elements related to the interfaces.

CHALLENGES FACING REQUIREMENTS ENGINEERS DURING THE ELICITATION PROCESS

As described previously, the requirements elicitation phase is characterized by a close interaction with customers, system users and others involved with or affected by the system. Accordingly, the gathered requirements are commonly in a form of written natural language so that this form of human-centric representation is understood by both customers and developers. However, a written natural language requirement is commonly error prone and vague (Kamalrudin et al., 2011).

A number of requirements elicitation problems are the problems of scope, the boundary of the system is ill-defined, unnecessary design information may be given, problems of understanding, users have incomplete understanding of their needs, users have poor understanding of computer capabilities and limitations, analysts have poor knowledge of problem domain, user and analyst speak different languages, ease of omitting "obvious" information, and conflicting views of different users (Christel et al., 1993; Sutcliffe et al., 2013).

A number of methods have been developed to address the above mentioned problems and improve the elicitation of requirements. For example, the ORDIT methodology (Blyth et al., 1993) emphasizes the definition of organizational requirements as part of the elicitation process. Accordingly, system designers can reason about organizational goals, policies and structures. The authors also developed a language with which to discuss human requirements of socio-technical systems, and to demonstrate how these are linked to the technical features of the system design.

The AMORE project (Christel et al., 1993) is concerned with ways in which large amounts of multimedia information can be visualized, stored and retrieved. AMORE is a system that provides ways to capture and organize requirements generated in many formats to facilitate navigation and browsing of large quantities of material.

Other methodologies that were developed and/or proposed to improve requirements elicitation include concepts like maps and repertory grids (Shaw et al., 1996)

and ethnographic techniques (Sommerville et al., 1994).

Amore recent method to improve requirements elicitation is available in Konaté et al (2014) and Sakamoto et al. (2014) where the authors have proposed a method to elicit functional requirements based on existing products that are available in repositories. They accordingly used mobile applications available on the mobile app stores to elicit requirements under an interaction perspective. Other recent work included proposing and validating a framework to help requirements engineers select the most adequate elicitation techniques at any time (Carrizo et al., 2014), while in Konaté et al. (2014) the authors adapted the separation of concerns method to focus on collaborative aspects of requirements elicitation. They separated engineering aspects from collaboration aspects in order to study both of these aspects and then integrate them.

Jin et al. (2003) presented an approach to support the elicitation process which combines various techniques for requirements elicitation including model-based concept acquisition, goal-driven structured interview and concept reuse, their goal was to support the automation of interaction with customers and to automate the construction of requirements models.

Our proposed framework for improving requirements elicitation addresses the problems of scope and completeness, were we aim to help analysts to be able to bound the system scope and ensure collecting the system requirements even those which are implicit and could be missed out.

Accordingly, our framework is expected to resolve many of the current requirements elicitation problems, such as identifying the system boundaries and including all relevant questions during interviews and questionnaires.

THE NEW REQUIREMENTS ELICITATION FRAMEWORK

Figure 1 represents our proposed framework process model, to elicit requirements starting from existing business process models.

Identifying the system's units of work (UoW) and functions to build the CRUD matrix

As we mentioned earlier, documents analysis is the first step to prepare for interviews or other interaction based elicitation techniques. The requirements of the new system can be easily elicited after understanding the existing system. In this phase we deploy a straightforward algorithm to extract essential business entities as well as functions for the as-is system from the system's business process model.

An Essential Business Entity (EBE) is part of the subject matter of the organization (Ould, 2005). So, EBEs

are there because of the business the organization is in, and they can be identified using a brainstorming exercise with the key person in an organization to answer questions concerned with what the organization makes and what product lines and/or service lines it has, what things the organization can be differentiated from other organizations in the same industry, what events in the outside world it needs to respond to, what business entities are listed in the organization's data model and what things do the organization's information systems keep information on. Recognizing who the organization's external and internal customers, can also help in identifying EBEs.

EBEs can be filtered by putting the word 'a' or 'the' in front of each suggestion. If it is not familiar, it should be excluded. Designed entities, which are there because of the way the organization chooses to do its business rather than because these entities characterize its business fundamentally, should also be excluded. For example, an "invoice" is not an EBE for a car manufacturer organization because it is not in the business of invoices. However, for the invoice handling department, which is in the business of handling invoices, an "invoice" is an EBE.

In this content we are concerned with EBEs which are considered as units of work (UoW). A Unit of Work is an EBE which has a lifetime handled by members of the organization. For example, an EBE that does not have a lifetime of interest to the organisation, or that is part of another EBE. We will not also consider EBE that are only roles that play part in the processes (Ould, 2005).

In our framework, we adapt the method used to automatically identify EBE from the set of business process models presented in (Yousef, 2014) and extend it to identify functions and units of work and then build the CRUD matrix, Algorithms I and II.

Algorithm 1: Building the CRUD matrix from BPMs

Input: the set of business process models, $BP = \{bp_1, bp_2, \dots, bp_i, \dots, bp_n\}$, $0 \leq i \leq n$

Output: The CRUD matrix

Begin

1. For each business process model bp_i in BP do
2. Find irredundant tasks of bp_i , $T = \{t_1, t_2, \dots, t_k, \dots, t_x\}$, $0 \leq k \leq x$
3. Call *Extract_EBEs_from_BPMs* (bp_i), return $E = \{e_1, e_2, \dots, e_b, \dots, e_y\}$, $0 \leq i \leq y$
4. Classify EBEs that have a lifetime which is handled by, or are the responsibly of, members of the organisation as Units of Work (UoWs)
5. End for
6. Build the CRUD matrix as follows:
7. for each task t_k in T do
8. set the t_k as the row of the CRUD matrix
9. for each unit of work, u_j in UOW do:
10. set u_j as the column of the CRUD matrix

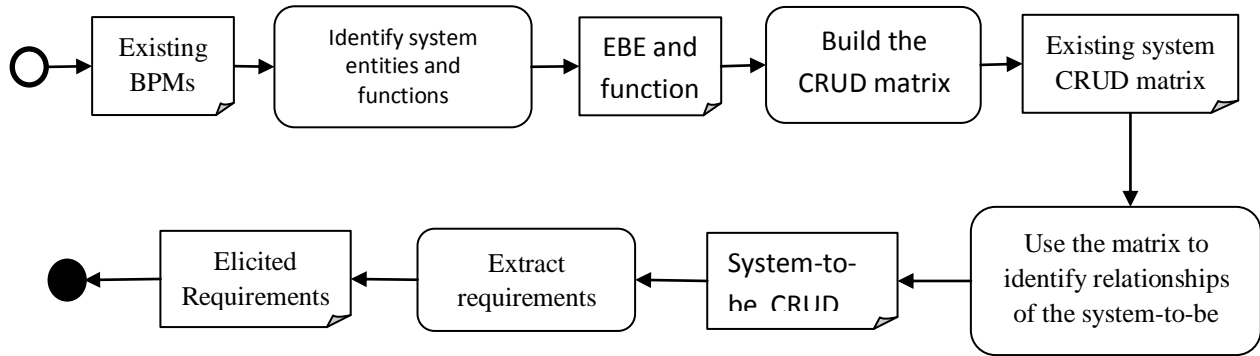


Figure 1. The proposed framework process model.

11. Set the matrix cell as the relationship between t_k and u_l which is one of the CRUD functions (Create, Read, Update or Delete)

12. end for

13. end for

End

Algorithm II: Extract_EBEs_from_BPMs

Input: A BPMN business process model, bpm_i , where it is a collection of roles, $R = \{r_0, \dots, r_m\}$ consisting of a number of tasks $T = \{t_0, \dots, t_j\}$

Output: A set of essential business entities present in the bpm_i , $EBE = \{ebe_0, \dots, ebe_{x1}\}$

Begin

Add the bpm_i process name to EBE;

Identify the set of rolls in the business process model, bpm_i ; $R = \{r_0, r_1, \dots, r_j, \dots, r_n\}$, $0 \leq j \leq n$;

For each role r_j in R do the following

Add r_j role name to EBE

Identify the set of tasks in r_j , $T = \{t_0, t_1, \dots, t_k, \dots, t_m\}$, $0 \leq k \leq m$;

For each task t_k in T do the following

If not already in EBE

Add the subject name of the task t_k to

EBE

Add the object name of the task t_k to EBE

End if

End for each task

Exclude designed business entities

End for each role

End

Algorithm I analyses each business process model to extract existing tasks and entities, where each irredundant task is placed in one row and each unit of work, identified as an essential business entity which has a lifetime managed by the organization (Ould, 2005), is placed in a column to build the CRUD matrix's headers. Algorithm II (Yousef, 2014) assumes that the business

processes are modelled using BPMN, however, this algorithm can be generalized for any business process model using model translators such as Yousef et al. (2009).

As we have explained in previously, brainstorming is conducted with members from different departments and domain experts to improve thinking by helping to answer specific questions. So, a brainstorming session is required at this stage to set the matrix's cell relationships for the as-is system.

Building a potential CRUD matrix and eliciting requirements

Having the CRUD matrix built, the system boundaries are set and all potential questions can be extracted from this matrix. According, the analyst can use this matrix to set new relationships for the new system, and generate a new one for the new system. Both matrices will help the analyst phrase questions with a minimum chance of missing a functionality of the system. This would lead to an improvement of the requirements' completeness to a great extent. Following we demonstrate our framework using a real case study form the healthcare domain.

DEMONSTRATING THE PROPOSED FRAMEWORK USING A CASE STUDY

Here, we demonstrate the new framework using a real case study from the healthcare domain; the Cancer Care and Registration in Jordan (CCR).

The Jordan's Cancer Care and Registration (CCR) processes' case study (AbuRub, 2006) is a real case study that has been validated and improved. It provides a number of business process models (BPM)s which we have used to demonstrate the new requirements elicitation framework.

Figure 2 shows one of the CCR processes; the patient reception process. This process was originally obtained

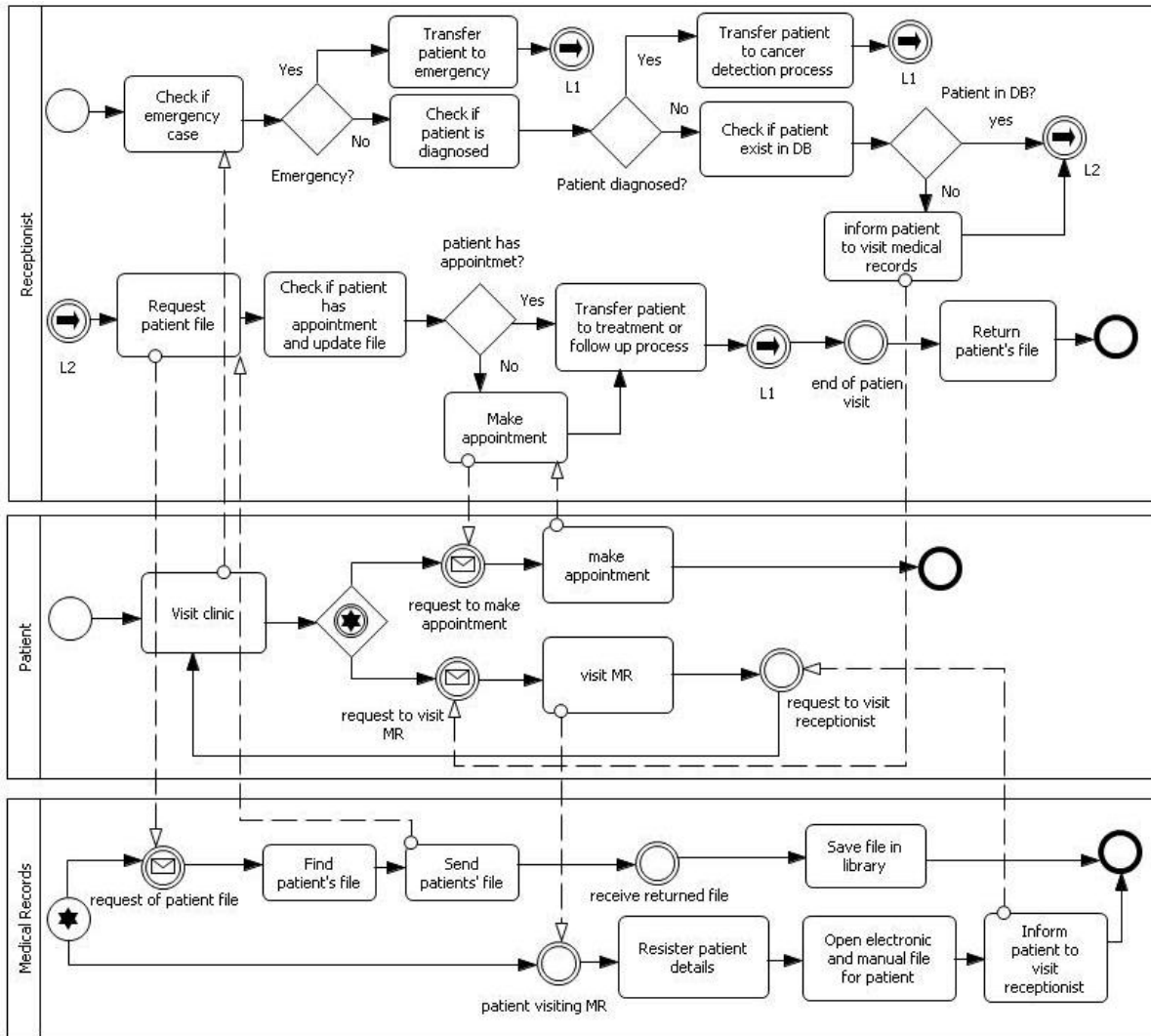


Figure 2. The Patient's Reception Process of the CCR case Study (AbuRub, 2006; Yousef et al., 2009).

from (AbuRub, 2006) and then translated from Role Activity Diagram to Business process model notation diagram by Yousef et al. (2009).

Identifying EBE

A set of EBE were identified using Algorithm II, these are listed in Table 1. Then the set is filtered to produce the Units of Work (those in boldface).

Identifying tasks and building the CRUD matrix

The set of tasks were extracted from the business process model, shown in Table 2, and the CRUD matrix was built using Algorithm I, Figure 3 shows part of the generated CRUD matrix.

The generated CRUD matrix provides an inspiring source of information to the analyst to phrase interview questions and questionnaires, to brainstorm potential relationships between entities and functions, etc. for example, the analyst can identify new functional requirements established from new compensations between entities and functions such as "informing patient" and "appointment", where no relation had been established in this context.

Accordingly, this matrix which was generated from analyzing existing documents, more specifically, the business process models, helps determine the system's boundaries and improves requirements' completeness to a great extent.

CONCLUSIONS

Traditional requirements elicitation techniques include

Table 1. The set of identified EBEs for the CCR case study; the patient's reception process (UoW in boldface).

Reception	Receptionist
Patient	Medical record
Emergency case	Emergency department
Diagnosis	Cancer detection
Database	Electronic File
Appointment	Treatment
Follow-up	Manual file

Table 2. The set of identified functions (tasks).

Check if emergency case	Transfer patient to follow-up
Transfer to emergency	Return patient file
Check if patient diagnosed	Visit clinic
Transfer patient to cancer detection	Visit mr
Check patient Id in DB	Find patient file
Inform patient to visit medical records	Send patient file
Request patient file	Register patient details
Check if patient has appointment	Save file
Update file	Open electronic file
Make appointment	Open manual file
Transfer patient to treatment	Inform patient to visit receptionist

functions	Entities	Medical record	Emergency case	Emergency department	Diagnosis	Electronic File	Appointment	Treatment	Follow-up	Manual
check if emergency case		R	R	R						
transfer to emergency		U	C	C						
check if patient diagnosed		R			R	R				R
transfer patient to cancer detection		U				U	C			U
check id patient in DB		R								
inform patient to visit medical records										
request patient file						R				R
check if patient has appointment		R					R			
update file		U				U				U
make appointment		U				C	U			U
transfer patient to treatment		U				U	C			U

Figure 3. Part of the CRUD matrix for the as-is system.

interviews, questionnaires observation, brainstorming, focus group, prototyping, document analysis, requirements workshop (joint application development, JAD) and interface analysis. Many requirements elicitation problems has been identified in literature, these include the problems of scope, system boundary, understand-ability, poor knowledge of problem domain different terminologies between users and analysts and conflict views of different users. In this paper we were concerned about improving requirements elicitation to help analysts understand the boundaries and identify all entities and functions of the as-is system, so that better questions are phrased during interviews and questionnaires. The proposed framework starts with an inception phase to establish the business case, where in almost all successful applications' developments; this phase constitutes a major part of the development process. Then the framework builds the CRUD matrix of the as-is system, from which requirements of the new system can be inspired. As a consequence, the proposed framework improves requirements' completeness to a great extent. The framework was demonstrated using a real case study from the healthcare domain, the cancer care and registration in Jordan. The next phase in our framework development is to implement a tool to automatically generate questions from the CRUD matrix formulated from the entities identified in the first phase of our framework. So, an analyst can have a well-structured interview with the set of questions that include all entities and functions within the system boundaries.

Conflict of Interest

The authors have not declared any conflict of interest.

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