Biological and Clinical Sciences Research Journal
eISSN: 2708-2261; pISSN: 2958-4728
www.bcsrj.com
DOI: https://doi.org/10.54112/bcsrj.v2022i1.159
*Biol. Clin. Sci. Res. J., Volume, **2022**: 159*
Original Research Article

EFFECT OF DIFFERENT MEDIA COMPOSITIONS OF 2,4-D, DICAMBA, AND PICLORAM ON CALLUS INDUCTION IN WHEAT (*TRITICUM AESTIVUM* L.)

ASHRAF A[1], AHMED N[1], SHAHID M[2],*ZAHRA T[3], ALI Z[4], HASSAN A[5], AWAN A[5], BATOOL S[5], RAZA MA[5], IRFAN U[5], MAQSOOD Z[5], MAKHDOOM M[6], *KHALID MN[5]

[1]*Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture Faisalabad, Pakistan*
[2]*Cotton Research Institute Multan, Pakistan*
[3]*Department of Botany, University of Agricultural Faisalabad, Pakistan*
[4]*Department of Agronomy, University of Agriculture Faisalabad, Pakistan*
[5]*Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan*
[6]*Wheat Research Institute, Ayub Agriculture Research Institute Faisalabad, Pakistan*
**Correspondence author email address:* tafseerzahra721@gmail.com, noumankhalidpbg@gmail.com

(Received, 7th September 2022, Revised 15th December 2022, Published 17th December 2022)

Abstract: *Wheat is a major cereal crop grown worldwide. Genus Triticum contains many species, among which T. aestivum has commonly grown, also known as bread wheat. Its genome is hexaploid, containing 42 chromosomes (2n=6x=42). To improve the response of wheat cultivars against biotic and abiotic stresses, it is genetically engineered with different biotechnological tools. Tissue culture technology has been proved vital for improving crop species in different quality and yield-related traits. Being a monocotyledons plant, wheat shows recalcitration toward tissue culture. The response of wheat to regeneration can be improved by using different growth-promoting hormones. The proposed study's objective was to increase callus induction's efficiency proposed study was to increase efficiency. The proposed study's objective was to increase callus induction's efficiency using different growth regulators treatments. MS media with varying concentrations of 4, 8, and 12 mg/L of dicamba, 2, 4-D, and picloram was used to check their effect on callus induction in common wheat cultivars Anaj-2017 and Akbar-2019. Media complemented with picloram at 8 mg/L was the supreme efficiency. About 511 mg and 420 mg of callus formation were observed at 8 mg/L for picloram succeeded by dicamba which was 340 mg and 350 mg at 12 mg/L, and then by 2, 4-D, which was 112 mg and 236 mg at 8 mg/L from matured embryos of Anaj-2017 and Akbar-2019 respectively.*

Keywords: wheat, tissue culture, callus induction

Introduction

Most of the world's population utilizes wheat (*Triticum aestivum*) as an essential and foremost cereal crop. About 36% of the world's population (or 2 billion people) use wheat as a daily food intake. About 55% of carbohydrates and 20% of diet calories are supplied by wheat worldwide (Breiman and Graur, 1995). Wheat belongs to the grass family named Poaceae (previously called Gramineae). Triticeae (also known as Hordeae) tribe comprises above 15 genera and 300 species comprising wheat, barley, oat, rye, maize, and rice (Briggle and Reitz, 1963). Wheat is divided into three types for genome sets wiz, diploid, tetraploid, and hexaploid, containing 14, 28, and 42 chromosomes, respectively, with basic chromosome number x = 7. The most commonly utilized wheat is the bread wheat (*T. aestivum*). At the same time, the major species include *T. durum* and *T. compactum* derived from the natural hybridization of ancestral wheat varieties, which are no longer cultivated nowadays (Briggle, 1967).The development of inflorescences, especially the initiation stage of spikelets and flowers, is important for the production and survival of flowers and, ultimately, for the maximum production aptitude. Despite the significance, molecular research on the inflorescences of wheat has been restricted until now due to the absence of high-quality genome reference genome sequences (Kippes *et al.*, 2014). So far, it has been found that only a few genes are associated with the development of spikes in wheat (Boden *et al*., 2015). There is an urgent need for methodological learning of the genes and their regulatory networks for the development of a wheat spike. The transcriptional profiling of various key stages in the development of wheat inflorescence must offer a new appreciation of the molecular technique involved in the development of wheat inflorescence. With the advancement in the sequencing of the genome of wheat and the emergence of better genome sequencing, additional

[Citation: Ashraf, A., Ahmed, N., Shahid, M., Zahra, T., Ali, Z., Hussain, A., Awan, A., Batool, S., Raza, M.A., Irfan, U. Maqsood, Z., Makhdoom M., Khalid, M.N., (2022).Effect of different media compositions of 2,4-d, dicamba, and picloram on callus induction in wheat (*Triticum aestivum* L.). *Biol. Clin. Sci. Res. J.*, **2022***: 259.* doi: https://doi.org/10.54112/bcsrj.v2022i1.159]

queries may be asked about the mechanisms of gene regulation for the development of an ear in the hexaploid species of wheat, such as the relationship among various homeologs and the general expression level of entire homeologs of important controlling genes contributing to the absolute output. Hexaploid wheat contains seven groups of chromosomes, each consisting of six chromosomes derived from three ancestral wild genomes of wheat, thus comprising a total of 21 pairs (or 42 chromosomes), making common wheat hexaploid wheat **(AABBDDGG).** Based on their genetic order similarity, during meiosis, these 21 chromosome pairs combine through centromere when they start clustering in hexaploid or tetraploid wheat. As meiosis progresses, multiple chromosomes from different genomes are associated with a single genome. At later stages of meiosis, seven clusters of chromosomes formed with multiple associations forming hexaploid or tetraploid wheat. There are multiple chances for chromosomes from different genomes to associate with other chromosomes. But this confusing situation is resolved through the action of loci of different chromosomes, arranging them as homologous chromosomes as meiosis proceeds to complete (Leach *et al.*, 2014).

Plant tissue culturing is a vast field that includes the culturing of any part of a plant, e.g., organs, tissues or cells, etc. Tissue culturing is done in an aseptic environment, incustomized environments, and in artificial media comprising entirely the compulsory nutrients and elements needed for the proper development of a plant or any organ of a plant. Callus induction is one of the biotechnological tools with which we can transform wheat for its better performance (Alikina *et al*., 2016). We can improve the performance of wheat through genetic transformation by analyzing the effect of various compositions of media cultures (Karsai *et al*., 1994). Plant regeneration via callus induction in wheat is mainly affected by the concentration of media, type of cultivar, and explants source. In the process of improving wheat cultivars through transformation, genetic engineering can be very effective. The initiation of the callus and the regeneration of the seedlings in wheat can be done by various types of explants. Tissue responses varied during *in vitro* seedling regeneration (Delporte *et al*., 2001). Embryos have been used for callus induction or DNA delivery methods in wheat plants (Chawla, 2002). Some types of explants have played a vital part in the regeneration method in culture. Regeneration of a wheat plant using a stem was twice that of a rachis plant (Majewsla *et al.*, 2007). The best-known method to conquer various practical problems is the cultivation of embryos *in vitro* (Dunwell, 1986).

Materials and methods

The proposed research was carried out at the Molecular Biology Laboratory, Centre of Agriculture Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad (UAF).

Explant preparation

Ripe caryopses of the wheat (*Triticum aestivum* L.) varieties Anaj-2017 and Akbar-2019 were used as a source of explant for *in vitro* mature embryo culture. The seeds were collected from the Ayyub Agricultural Research Institute (AARI) Wheat department, Faisalabad. The seeds were disinfected in 75% v/v ethanol for 3 minutes and washed five times with sterile and deionized water. The seeds were again dipped in 5.25% sodium hypochlorite with 2-3 drops of Tween 20 for 20 minutes, followed by washing five times with deionized sterile water. Surface sterilized seeds were placed in 100 × 15 mm Petri plates (on filter paper soaked with sterile water).

Preparation of MS Media

The working area should be designed to facilitate cleaning and reduce the possibility of contamination

Fig. 1: Equipment and chemicals for preparing MS media

An appropriate process for preparing the culture medium was to prepare concentrated stock solutions that can be diluted to the required concentration before use. Stock solutions of macro-nutrient were prepared 10 times stronger than the working solutions. Micro-nutrient stock solutions were 100 times more concentrated than those required for working solutions. The stock solution was kept in the refrigerator until used.

[Citation: Ashraf, A., Ahmed, N., Shahid, M., Zahra, T., Ali, Z., Hussain, A., Awan, A., Batool, S., Raza, M.A., Irfan, U. Maqsood, Z., Makhdoom M., Khalid, M.N., (2022).Effect of different media compositions of 2,4-d, dicamba, and picloram on callus induction in wheat (*Triticum aestivum* L.). *Biol. Clin. Sci. Res. J.,* **2022***: 259.* doi: https://doi.org/10.54112/bcsrj.v2022i1.159]

Table 1: Ingredients used for the preparation of MS media along with their chemical formula and concentrations.

Sr. #	Chemical	Formula
Macronutrients		
1	NH4NO3	Ammonium nitrate
2	KNO3	Potassium Nitrate
3	CaCl2.2H2O	Calcium chloride
4	MgSO4.7H2O	Magnesium sulphate
5	KH2PO4	Potassium-dihydrogen-orthophosphate
Micronutrients		
6	KI	Potassium Iodide
7	H3BO3	Boric Acid
8	MnSO4.4H2O	Manganese sulphate
9	ZnSO4.7H2O	Zinc Sulphate
10	Na2MoO4.2H2O	Sodium molybdate
11	CuSO4.5H2O	Cupric sulphate
12	CoCl2.6H2O	Cobaltous chloride
Vitamins		
13	C6H12O6	Myo-Inositol
14	C2H5NO2	Glycine
15	C12H18Cl2N4OS	Thiamine HCl
16	C8H11NO3	Pyridoxine HCl
17	C6H5NO2	Nicotinic acid
Iron source		
18	Na2EDTA	
19	FeSO4.7H2O	lFerrous sulphate
20	C12H22O11	Sucrose
21		Agar

Callus induction media Preparation

Callus induction media was prepared by adding MS (Murashige and Skoog, 1962) basal salt mixture in water which was supplemented with different growth regulators 2, 4-D, dicamba, and Picloram that was 4 mg/L, 8 mg/L, and 12 mg/L. Anaj-2017 and Akbar-2019 were the cultivars of wheat evaluated with these three concentrations of growth regulators. In addition to growth regulators, stock solutions of vitamins and iron were also prepared. Effects of different concentrations of 2, 4-D, dicamba, and Picloram were observed on the type of callus produced were also determined visually. For the preparation of 1L MS media 10ml of Vitamin from the stock solution, 10ml of Iron solution and 30g of Stock solution of the vitamin was prepared using nicotinic acid, glycine, myo-inositol, thiamine HCL and pyridoxine. Sodium Versinate was used for the preparation of the iron stock solution. Concentrations of these chemicals are described in (Table2). Media was prepared, autoclaved and then poured into the plates by taking all precautionary measures. After sucrose were added in the media. To solidify the callus induction media, 10g of agar was used. The pH of the callus induction media was maintained at 5.8.

Table 2: Stock solutions of vitamins and Iron.

Vitamin stock solution	
Nicotinic acid	10mg/100ml
Glycine	100mg/100ml
Myo-Inositol	1g/100ml
Thiamine HCL	10mg/100ml
Pyridoxine	10mg/100ml
Iron stock solution	
Sodium versinate215mg/50ml	

pouring, the media was allowed to solidify at room temperature. Plates with solidified media were incubated at room temperature for 7 days to check for contamination. Media that showed no contamination were used to conduct further experiments.

[Citation: Ashraf, A., Ahmed, N., Shahid, M., Zahra, T., Ali, Z., Hussain, A., Awan, A., Batool, S., Raza, M.A., Irfan, U. Maqsood, Z., Makhdoom M., Khalid, M.N., (2022).Effect of different media compositions of 2,4-d, dicamba, and picloram on callus induction in wheat (*Triticum aestivum* L.). *Biol. Clin. Sci. Res. J.*, **2022***: 259.* doi: https://doi.org/10.54112/bcsrj.v2022i1.159]

Figure: 2 Preparation material of Callus

Induction Media

Callus induction

Endosperm and radicals were removed from the caryopsis of aseptically disected mature wheat embryos for embryo isolation. About 1-2 mm of embryo sections were placed in callus-inducing media so that shoot tips, nodular parts and mesocotyl were positioned on the media surface. Six embryos were cultivated in 1 petri dish, and the embryo plate was considered an experimental unit. MS (Murashige and Skoog, 1962) media, along with inorganic nutrients, is a callus induction and maintenance medium supplemented with glutamine, glycine, myo-inositol, casein hydrolyzate, nicotinic acid, pyridoxine, and thiamine for 5.0 mg / L, 2.0 mg / L, 1.0 mg / L, 1.0 mg / L, 0.5 mg / L, 0.5 mg / L, and 0.1 mg / L respectively.

The embryos placed in the media showed enlargement within 2-3 days, and after almost one-week formation of callus from the embryos was initiated. To maintain enough supply of essential nutrients florcalli, these were transferred to fresh culture media after every two weeks.

Determination of callus growth rate:

Data was collected for callus growth rate, type of calli and mass at regular intervals. Callus induction frequency (%) was recorded at different intervals to evaluate the callus induction efficiency of mature embryos using concentrations of 2, 4-D, dicamba, and Picloram. Calli were transferred at different time intervals to a sterile petri dish, and the weight of calli in a plate was measured under aseptic conditions. After weighing, callus pieces were re-transferred to callus culture medium. After 6 weeks, data were recorded for the callus induction frequency of two wheat cultivars,Anaj-2017 and Akbar-2019, according to the formula.

$$\textbf{Callusinductlionfrequency}\ (\%) = \frac{\textbf{No. ofembryosproducedcalli}}{\textbf{No. ofembryocultured}} \times \mathbf{100}$$

Callus proliferation efficiency was also estimated by using the following equation.

$$\textbf{Proliferationefficiency}\ (\%) = \frac{\textbf{No. ofproliferatedcalli}}{\textbf{No. ofincubatedembryos}} \times \mathbf{100}$$

Maintenance of induced calluses:

Dicamba, picloram, and 2, 4-D were three auxins used in experimentation. Each auxin was experienced at three varying compositions 4.0, 8.0, and 12.0 mg/L concentration. All organic components were taken from the lab. The whole media was polymerised/solidified with Agar at10 g/l, and pH was optimized to 5.7 with 1M NaOH. The media was autoclaved at 121ºC for about 15 minutes at 15 psi pressure. Petri dishes comprising 6 cultured embryos were wrapped with parafilm and placed at 25°C temperature in a dark environment. The

[Citation: Ashraf, A., Ahmed, N., Shahid, M., Zahra, T., Ali, Z., Hussain, A., Awan, A., Batool, S., Raza, M.A., Irfan, U. Maqsood, Z., Makhdoom M., Khalid, M.N., (2022).Effect of different media compositions of 2,4-d, dicamba, and picloram on callus induction in wheat (*Triticum aestivum* L.). *Biol. Clin. Sci. Res. J.*, **2022***: 259*. doi: https://doi.org/10.54112/bcsrj.v2022i1.159]

cultures were shifted to fresh medium with an interval of 2 weeks. Callus selection was not made at this stage. After about 8 weeks of culture initiation, the fresh callus mass was weighed and calculated by evaluating the complete mass of callus developed by each embryo.

Plantlet Regeneration:

After every 2 weeks, embryogenic calluses were shifted to a growth chamber at 27°C temperature, with a 16 hours light and 8 hours darkness period in Petri dishes on regeneration media (MSR) comprising 2,4-D, picloram, and dicamba. The development of green buds was seen in the first seven days and vegetative development within the first 3 weeks. The plant regeneration percentage was measured based on embryos regenerated from the number of embryos placed on callus induction media.

After 8 weeks of culture, embryos developed into calluses and were shifted to 100 to 25 mm Petri dishes containing regeneration media. The regenerated calluses were placed in a plant growth chamber at a temperature 25°C with appropriate light concentration made available by cold, fluorescent white tube light. Subcultures were repeated every 2 weeks. The total number of calluses regenerated per embryo was calculated after about 4 weeks of cultivation in the regeneration medium. Three types of auxins in 3 levels were used in this experimentation. Each six-embryo petri dish was considered as one experimental unit. Experiment was conducted in a completely randomized design with 5 replications. Callus fresh weight was taken from Petri plates to assess the response of growth-regulating hormones

Results and discussion

Callus Induction

Callus initiation and embryogenesis were observed in embryos cultured on all three media types. Approximately 100% of the developed embryos cultivated in medium with three auxins exhibited that callus development began within 3 days of inoculation. The average callus fresh weight per treatment varied from 25 to 270 mg per embryo.

$$\mathbf{Callusinductionfrequency}\ (\%) = \frac{\mathbf{No.ofembryosproducedcalli}}{\mathbf{No.ofembryocultured}} \times \mathbf{100}$$

Mainly, two kinds of calli have been seen in cultures: non-embryogenic and embryogenic calluses. Non-embryogenic calli were distinguished by their cream colour and soft, loose, and watery touch. The embryogenic calli were pale, smooth, and compact and comprised embryonic structures forming independent or bonded nodules. The embryogenic callisurrounded bynon-embryogeniccalliwere detected in most explants as shown in figure 3. Constant with previous findings related to calli developed from mature embryos of wheat, our results showed that many treatments had a higher rate of non-embryogenic callusMacKinnon*et al.*, 1987).

Fig. 3: Embryogenic and Non-Embryogenic calli.

The type and dose of auxin strongly influenced the callus development. A significant interaction was observed between the type of auxin used and auxin dose. The fresh callus weight increased by increasing the composition of picloram, and dicamba, but the fresh callus weight decreased by increasing the concentration of 2, 4-D. This adverse response of high levels of 2, 4-D was depicted by the necrotic brownish presence of callus experienced in approximately 92% of the cultures prepared from developed embryos with higher levels of 12 mg/L of 2, 4-D. The medium contained picloram was found to be the most efficient in encouraging callus development from mature embryos of Anaj-2017 and Akbar-2019, subsequently by dicamba, and then for 2, 4-D. Zhou and Lee (1984) reported the same results using developed embryo cultures of the Chinese Spring variety. However, in this study, dicamba improves callus development compared to picloram.

Contrary to this, picloram, dicamba and 2, 4-D respond similarly in winter wheat 'Frederick'. Genotypes correspond differently *in vitro* conditions(Özgen *et al.*, 1998). Dicamba and Picloram have higher callus-producing capability than 2, 4-D in spring wheat (Papenfuss and Carman, 1987; Carman, 1988) and in winter wheat cultivars(Hunsinger and Schauz, 1987; Carman, 1988).The best, average and good quality of callus were observed in this study, as shown in figure 4.

[Citation: Ashraf, A., Ahmed, N., Shahid, M., Zahra, T., Ali, Z., Hussain, A., Awan, A., Batool, S., Raza, M.A., Irfan, U. Maqsood, Z., Makhdoom M., Khalid, M.N., (2022).Effect of different media compositions of 2,4-d, dicamba, and picloram on callus induction in wheat (*Triticum aestivum* L.). *Biol. Clin. Sci. Res. J.*, **2022***: 259.* doi: https://doi.org/10.54112/bcsrj.v2022i1.159]

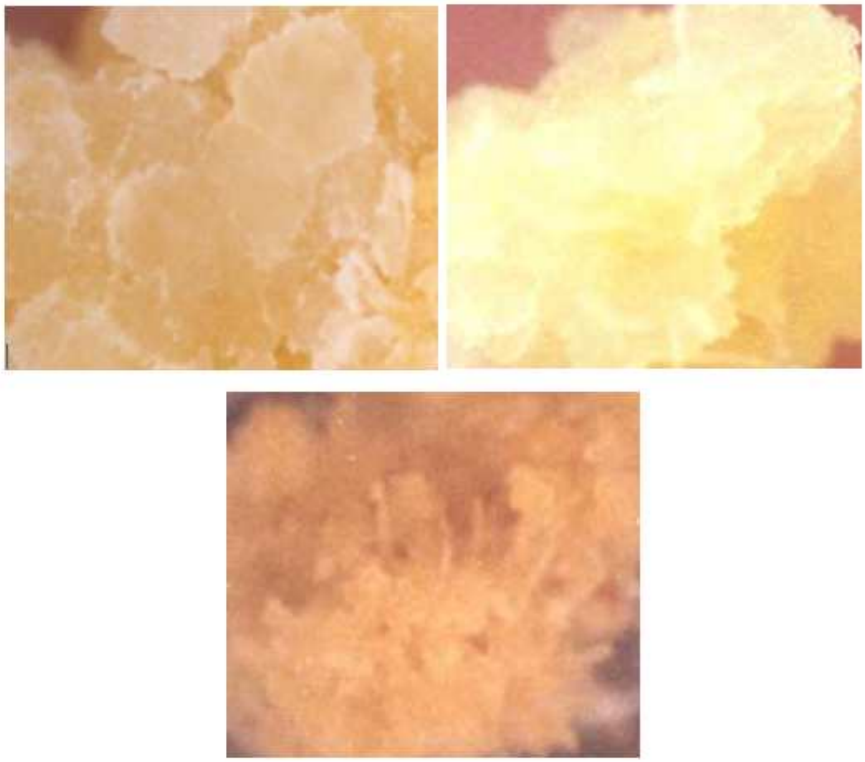

Fig. 4: Best, average, and good quality callus.

The fresh weight of callus was strongly influenced by the kind of auxin and concentration used. The mean weight of callus using 2, 4-D and its different levels are described in table 3

Table 3: Mean fresh weight of callus obtained from mature embryos of wheat grown in medium comprising 2, 4-D with three different levels

Treatments		Mean weight of induced calluses	
Auxin	**Auxin dose**	**Anaj-2017 (mg)**	**Akbar-2019 (mg)**
2,4-D	4.0	205	276

should be taken to improve such circumstances.

Conflict of interest

The authors declare no conflict of interest.

References

Aaronson, N. K., Ahmedzai, S., Bergman, B., Bullinger, M., Cull, A., Duez, N. J., Filiberti, A., Flechtner, H., Fleishman, S. B., and de Haes, J. C. (1993). The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. *JNCI: Journal of the National Cancer Institute***85**, 365-376.

Baffert, K.-A., Darbas, T., Lebrun-Ly, V., Pestre-Munier, J., Peyramaure, C., Descours, C., Mondoly, M., Latrouite, S., Bignon, E., and Nicouleau, S. (2021). Quality of life of patients with cancer during the COVID-19 pandemic. *in vivo***35**, 663-670.

Bargon, C., Batenburg, M., Van Stam, L., van der Molen, D. M., Van Dam, I., van der Leij, F., Baas, I., Ernst, M., Maarse, W., and Vermulst, N. (2020). The impact of the COVID-19 pandemic on quality of life, physical and psychosocial wellbeing in breast cancer patients–a prospective, multi-center cohort study. *European Journal of Cancer***138**, S17.

Chen, P., Mao, L., Nassis, G. P., Harmer, P., Ainsworth, B. E., and Li, F. (2020). Coronavirus disease (COVID-19): The need to maintain regular physical activity while taking precautions. *Journal of sport and health science***9**, 103.

Gallo, O., Bruno, C., Locatello, L. G., Martelli, F., Cilona, M., Orlando, P., Fancello, G., Maggiore, G., Viberti, F., and Ciabatti, P. (2021). The impact of the COVID-19 pandemic on the quality of life of head and

[Citation: Ashraf, A., Ahmed, N., Shahid, M., Zahra, T., Ali, Z., Hussain, A., Awan, A., Batool, S., Raza, M.A., Irfan, U. Maqsood, Z., Makhdoom M., Khalid, M.N., (2022).Effect of different media compositions of 2,4-d, dicamba, and picloram on callus induction in wheat (*Triticum aestivum* L.). *Biol. Clin. Sci. Res. J.*, **2022***: 259*. doi: https://doi.org/10.54112/bcsrj.v2022i1.159]

neck cancer survivors. *Supportive Care in Cancer***29**, 6297-6304.

Kutikov, A., Weinberg, D. S., Edelman, M. J., Horwitz, E. M., Uzzo, R. G., and Fisher, R. I. (2020). A war on two fronts: cancer care in the time of COVID-19. Vol. 172, pp. 756-758. American College of Physicians.

Lemon, J., Edelman, S., and Kidman, A. D. (2003). Perceptions of the "Mind-Cancer" relationship among the public, cancer patients, and oncologists. *Journal of Psychosocial Oncology***21**, 43-58.

Liang, W., Guan, W., Chen, R., Wang, W., Li, J., Xu, K., Li, C., Ai, Q., Lu, W., and Liang, H. (2020). Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. *The lancet oncology***21**, 335-337.

Qian, Y., Wu, K., Xu, H., Bao, D., Ran, F., Wei, W., Cheng, T., Huang, D., Lin, X., and Bruera, E. (2020). A survey on physical and mental distress among cancer patients during the COVID-19 epidemic in Wuhan, China. *Journal of palliative medicine***23**, 888-889.

Saini, K. S., de Las Heras, B., de Castro, J., Venkitaraman, R., Poelman, M., Srinivasan, G., Saini, M. L., Verma, S., Leone, M., and Aftimos, P. (2020). Effect of the COVID-19 pandemic on cancer treatment and research. *The Lancet Haematology***7**, e432-e435.

Scott, N. W., Fayers, P., Aaronson, N. K., Bottomley, A., de Graeff, A., Groenvold, M., Gundy, C., Koller, M., Petersen, M. A., and Sprangers, M. A. (2008). EORTC QLQ-C30 reference values manual.

Wang, H., and Zhang, L. (2020). Risk of COVID-19 for patients with cancer. *The Lancet Oncology***21**, e181.

Younger, E., Smrke, A., Lidington, E., Farag, S., Ingley, K., Chopra, N., Maleddu, A., Augustin, Y., Merry, E., and Wilson, R. (2020). Health-related quality of life and experiences of sarcoma patients during the COVID-19 pandemic. *Cancers***12**, 2288.

Yusuf, A. (2020). Cancer care in the time of COVID-19—a perspective from Pakistan. *ecancermedicalscience***14**.